Proceedings
SIXTIETH
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

HOTEL MORRISON
Chicago, Illinois
November 28-29-30, 1956
CONTENTS

SIXTIETH ANNUAL PROCEEDINGS

Photographs of Officers for 1956-1957........................................... vii
Officers and Committees 1957.................................................. ix
Record of Previous Meetings................................................ xiv
Welcome to Illinois Hon. Stillman J. Stanard.............................. 1
Response to Welcome H. U. Garrett........................................... 4
President's Address. A. L. Brueckner...................................... 6
Presentation of Key to A. L. Brueckner................................... 10
Report of Secretary-Treasurer Ralph A. Hendershott.................... 11
Report of Auditing Committee K. J. Peterson............................. 14
Memorial Service M. N. Reimenschneider.................................. 15
Pheasants O. Sussman et al................................................... 19
Report of Representative to Meeting of Commissioners, Directors and Secretary of Agriculture. A. P. Schneider.......................... 24
Report of Committee to ARS on Program & Budget W. L. Bendix........ 25
Report of Committee on Laws and Regulations. Ralph L. West......... 27
Report of Committee on Legislation. W. L. Bendix..................... 30
Report of Committee on Morbidity & Mortality. J. R. Hay.............. 33
Report of Committee on Public Relations. R. L. Knudson.............. 34
Report of Committee on Effect of Radioactive Material on Livestock. B. F.
Trum...................................................................................... 35
Report of Committee on Regulatory Education. W. R. Krill............. 37
Report of Committee on Resolutions. J. W. Hastings, Sr................ 39
Proposed Amendment to the Constitution and By Laws.................. 44
Report of Committee on Stockyards, Markets and Transportation. A. Z. Baker. 45

BILOGICS AND PHARMACEUTICALS

A Practical Evaluation of Live Immunizing Agents. J. M. Hejl........ 47
Report of Committee on Biologics and Pharmaceuticals. Hadleigh Marsh. 53

CATTLE

Anaplasmosis

Inhibition of Anaplasma Marginale Infection in Cattle with Oxytetracycline Hydrochloride. J. F. Christensen and J. B. Harrold................... 69
Report of the Committee on Anaplasmosis. K. J. Peterson et al........ 77

Brucellosis

State-Federal Cooperative Brucellosis Eradication Program. C. K. Mingle ... 82
Further Studies on Duration of Immunity to Brucellosis Induced in Calf-Vaccinated Cattle with Strain 19 Vaccine. E. R. Goode, C. A. Manthei, and T. E. Amerault................................. 89
The Effect of Bacterins Containing *Pasteurella Multocida* on Agglutinins for Brucella in Cattle. D. T. Berman ........................................... 97
Report of the Southern Conferences on Brucellosis. C. G. Scruggs .......... 104
Studies on a Differential Test for Nonspecific Brucella Agglutination Reactions in Bovine Serum. F. C. Stiles, T. G. White, F. C. Driver and M. H. Roepke .... 109
Report of the Committee on Brucellosis. R. W. Smith et al. ................ 119
Report Representative to National Brucellosis Committee. R. W. Smith ..... 131
*Bacillary Hemoglobinuria*
The Control of Bacillary Hemoglobinuria. L. Ds. Smith .................... 135

*Leptospirosis*

*Infectious Bovine Rhinotracheitis*
A Controlled Field Trial of a Vaccine for Infectious Bovine Rhinotracheitis. J. W. Kendrick, C. J. York and D. G. McKercher ................ 155
Report of the Committee on Infectious Diseases of Cattle. M. G. Fincher et al. ................................................. 159

*Tuberculosis*
Status of State-Federal Cooperative Tuberculosis Eradication. A. F. Ranney 163
Pathologic Studies on Tuberculin Reactors with No Visible Lesions. W. A. Anderson, W. T. Shalkop and A. B. Larsen ........................ 170
Report of the Committee on Tuberculosis. H. A. Milo et al. ................ 177

**EXOTIC DISEASES**
What Precautions are Taken to Prevent the Introduction of Foreign Animal Diseases. F. L. Herchenroeder .......................... 179
Report of the Committee on Exotic Diseases. F. A. Todd et al ............. 183

**PARASITIC DISEASES**
Sheep and Cattle Scabies Eradication. J. L. Hourrigan ....................... 186
Sheep Scabies Control in Ohio. J. E. Doran ................................ 191

**POULTRY DISEASES**
State Wide Testing for PPLO Infection of Poultry. W. R. Dunlop and R. G. Strout 197
Immunologic Differences in Strains of Infectious Bronchitis Virus. E. L. Jungherr, T. W. Chomiak and R. E. Lugenbuhl ......................... 203
Report of the Committee on Transmissible Diseases of Poultry. M. S. Hopstad et al .................................................. 210

**PUBLIC HEALTH**
Fifty Years of Federal Meat Inspection C. D. Van Houweling ..................... 224
## CONTENTS

### RABIES

State-Wide Rabies Control in Georgia 1946-1956 L. E. Starr, S. M. Canup, R. L. Watson and M. Jernigan .................................................. 231
Report of Committee on Rabies V. D. Chadwick et al. ......................... 256

### SWINE DISEASES

Twelve Years’ Successful Vaccination of Farm Herds. C. G. Cole, J. P. Torrey and M. E. Zinober. ................................................. 263
Report of the Committee on Nationwide Eradication of Hog Cholera J. Miligan et al .............................................................. 270
Report of Committee on Transmissible Diseases of Swine. H. U. Garrett et al .......................................................... 283
Report of Committee on Swine Brucellosis R. W. Carter et al ............. 287

### VESICULAR DISEASES

**Vesicular Stomatitis**

Enzootic Vesicular Stomatitis. R. P. Hanson and L. Karstad .................. 288
A Typing Study of Vesicular Stomatitis Virus Freed Samples of Swine Origin. A. A. Holbrook, J. N. Geleta and W. C. Patterson ............... 293
Recovery of New Immunological Types of Vesicular Exanthema Virus. R. A. Baukowski ................................................................. 302
Nutritive Value and Chemical Analysis of Garbage Fed on New Jersey Swine Farms. C. A. Cabell ....................................................... 321
The Differentiation of Vesicular Disease by Serological Procedures. C. E. Rice ................................................................. 325
Report of the Committee on Nominations. C. E. Kord et al. .................. 335
Election and Induction of Officers for 1957 .................................... 335
Adjournment .............................................................................. 336
Constitution & By Laws .................................................................. 337
61st Meeting November 13-14-15 1957. Chase Park Plaza Hotel, St. Louis Missouri ................................................................. Cover 4
OFFICERS 1956–1957

G. H. GOOD
President

J. MILLIGAN
1st Vice-President

R. A. HENDERSHOTT
Secretary-Treasurer

H. U. GARRETT
2nd Vice-President
Deceased

F. G. BUZZELL
3rd Vice-President
OFFICERS AND COMMITTEES FOR 1956-57

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H. U. Garrett 2nd Vice President ............................. Des Moines, Iowa
F. G. Buzzell 3rd Vice President ............................. Augusta, Maine

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M. Welsh, Princeton, New Jersey
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R. A. Hendershott, Trenton, New Jersey
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F. J. Mulhern, Falls Church, Virginia
A. H. Quin, Kansas City, Missouri
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K. J. Peterson, Salem, Oregon
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AGRICULTURE MARKETING SERVICE
A. L. Brueckner, Baltimore, Maryland

REPRESENTATIVE TO NATIONAL BRUCELLOSIS COMMITTEE
R. W. Smith, Concord, New Hampshire

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DIRECTORS, AND COMMISSIONERS OF AGRICULTURE

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R. W. Smith  D. L. Haley
W. L. Bendix  J. V. Smith

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R. L. Cuff, Kansas City, Missouri  B. Schwartz, Washington, D. C.
J. Hourrigan, Washington, D. C.  L. E. Swanson, Gainesville, Florida

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J. P. Delaplane, College Station, Texas  P. P. Levine, Ithaca, New York
H. M. DeVolt, College Park, Maryland  J. W. Munn, Atlanta, Georgia
E. M. Dickenson, Corvallis, Oregon  N. O. Olson, Morgantown, West Virginia
J. F. Witter, Orono, Maine  B. S. Pomeroy, St. Paul, Minnesota
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G. H. Good, Cheyenne, Wyoming             O. Sussman, Princeton, New Jersey
H. J. Rollins, Raleigh, North Carolina    F. P. Wilcox, Los Angeles, California

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W. Jenkins, Pearl River, New York         L. A. Rosner, Jefferson City, Missouri
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W. R. Krill, Columbus, Ohio               I. A. Merchant, Ames, Iowa
R. S. Sugg, Auburn, Alabama

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L. L. Breeck, Frankfort, Kentucky          R. W. Carter, Columbia, South Carolina
J. S. Campbell, Little Rock, Arkansas     W. F. Fisher, Reno, Nevada
T. J. Grennan, Providence, Rhode Island
## Record of Previous Meetings

<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sept. 27-28, 1897†</td>
<td>Fort Worth, Texas</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. D. O. Lively, Fort Worth, Texas</td>
</tr>
<tr>
<td>2. Oct. 11-12, 1898</td>
<td>Omaha, Nebraska</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. Taylor Riddle, Kansas</td>
</tr>
<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, New York</td>
<td>Dr. E. P. Niles, Virginia</td>
<td>Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>6. Sept. 22-23, 1902</td>
<td>Wichita, Kansas</td>
<td>Mr. W. H. Dunn, Tennessee</td>
<td>Mr. Wm. P. Smith, Monticello, Illinois</td>
</tr>
<tr>
<td>8. Aug. 23-24, 1904</td>
<td>St. Louis, Mo.</td>
<td>Dr. J. C. Norton, Arizona</td>
<td>Mr. Wm. P. Smith, Monticello, Illinois</td>
</tr>
<tr>
<td>14. Dec. 5-6-7, 1910</td>
<td>Chicago, Ill.</td>
<td>Dr. C. E. Cotton, St. Paul, Minn.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>15. Dec. 5-6, 1911</td>
<td>Chicago, Ill.</td>
<td>Dr. John F. Devine, Goshen, N. Y.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>16. Dec. 3-4-5, 1912</td>
<td>Chicago, Ill.</td>
<td>Dr. Maryeck P. Ravenel, Madison, Wis.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>17. Dec. 2-3-4, 1913</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter F. Bahnson, Atlanta, Ga.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>22. Dec. 2-3-4, 1918</td>
<td>Chicago, Ill.</td>
<td>Dr. M. Jacob, Knoxville, Tenn.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>25. Dec. 6-7-8, 1922</td>
<td>Chicago, Ill.</td>
<td>Dr. W. E. Crews, Bismarck, N. Dak.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>27. Dec. 3-4-5, 1924</td>
<td>Chicago, Ill.</td>
<td>Dr. W. J. Butler, Helena, Montana</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>31. Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Es, Lincoln, Nebraska</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>32. Dec. 5-6-7, 1928</td>
<td>Chicago, Ill.</td>
<td>Dr. C. A. Cary, Auburn, Alabama</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>33. Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>34. Dec. 3-4-5, 1930</td>
<td>Chicago, Ill.</td>
<td>Dr. A. E. Wight, Washington, D. C.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>35. Dec. 2-3-4, 1931</td>
<td>Chicago, Ill.</td>
<td>Dr. J. W. Conaway, Columbus, Mo.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>37. Dec. 6-7-8, 1933</td>
<td>Chicago, Ill.</td>
<td>Dr. E. T. Faulder, Albany, N. Y.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>No.</td>
<td>Date</td>
<td>City, State</td>
<td>Members</td>
</tr>
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<tr>
<td>38</td>
<td>Dec. 5-6-7, 1934</td>
<td>Chicago, Ill.</td>
<td>Dr. T. E. Robinson, Providence, R. I.</td>
</tr>
<tr>
<td>39</td>
<td>Dec. 4-5-6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. Edward Records, Reno, Nevada</td>
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<tr>
<td>40</td>
<td>Dec. 2-3-4, 1936</td>
<td>Chicago, Ill.</td>
<td>Dr. Walter Wisnicky, Madison, Wisc.</td>
</tr>
<tr>
<td>43</td>
<td>Dec. 6-7-8, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
</tr>
<tr>
<td>44</td>
<td>Dec. 4-5-6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyoming</td>
</tr>
<tr>
<td>45</td>
<td>Dec. 3-4-5, 1941</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass.</td>
</tr>
<tr>
<td>46</td>
<td>Dec. 2-3-4, 1942</td>
<td>Chicago, Ill.</td>
<td>*Dr. I. S. McAdory, Auburn, Alabama</td>
</tr>
<tr>
<td>47</td>
<td>Dec. 1-2-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
</tr>
<tr>
<td>48</td>
<td>Dec. 6-7-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
</tr>
<tr>
<td>49</td>
<td>Dec. 5-6-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
</tr>
<tr>
<td>50</td>
<td>Dec. 4-5-6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N. Carolina</td>
</tr>
<tr>
<td>51</td>
<td>Dec. 3-4-5, 1947</td>
<td>Chicago, Ill.</td>
<td>Mr. Will J. Miller, Topeka, Kansas</td>
</tr>
<tr>
<td>53</td>
<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. T. O. Brandenburg, Bismarck, N. D.</td>
</tr>
<tr>
<td>57</td>
<td>Sept. 23-24-25, 1953</td>
<td>Atlantic City, N. J.</td>
<td>Dr. T. Childs, Ottawa, Canada</td>
</tr>
<tr>
<td>58</td>
<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W. Va.</td>
</tr>
<tr>
<td>59</td>
<td>Nov. 16-17-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Montana</td>
</tr>
<tr>
<td>60</td>
<td>Nov. 28-29-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
</tr>
</tbody>
</table>

* Deceased.
† This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
‡ Reprinted in 54th Annual Report.
Mr. President and Gentlemen of the United States Livestock Sanitary Association:

I do not feel particularly strange here this morning. My mind goes back to the year 1927, the last time I appeared before this Association, at which time I shared in a report to you on certain research matters which we had conducted on the possibility of immunization against tuberculosis. At that time we reported that our experimentation had been a complete failure. We had proven that the vaccines used from this country and from the Pasteur Institute in France were both of no value. Perhaps that conclusion was of some value.

My early connection with you gentlemen and your work goes back a good deal further than that, and I am dating myself when I admit at the outset that I have reached the age of maturity, the great age of 64. As Superintendent of the Dairy Division of the State of Illinois in the year 1918, I became connected with the State for a period of three months, at which time I made a survey of all the State institution herds. I determined then that those herds were full of tuberculosis and a disease that we called infectious abortion. I made certain recommendations at that time. That goes back quite a few years, and some of you boys won't quite remember what was going on in those days.

I soon became acquainted with the officials of various states in livestock disease control. I became acquainted with the officials of the United States Department of Agriculture back in the days when Dr. John R. Mohler and his associates were running things at the Bureau of Animal Industry. I largely respected them and their ability.

I want to impress upon you, if I make no other impression, that your industry, and the work being done in America today is resting upon a sound and solid foundation, a foundation established by men of the past, most of whom have gone to their rewards by this time, men who were dedicated to their jobs, who did a grand job in livestock disease control and in the research work that was necessary in connection therewith.

We should never forget the foundation upon which we built and on which we work. The other day I heard an address by a very fine lady, who spoke before a group of graduating nurses, and who said that those young ladies were much smarter than the nurses of the past—that they were much smarter even than Florence Nightingale. I was slightly nauseated. Florence Nightingale laid the foundation for all nursing, for all the work that has been done in over 100 years. The foundation was laid so that those young ladies who were graduating the other day might learn. Let us never disrespect the foundation that is laid for us to build upon.

When I appeared before you twenty-nine years ago—and, by the way, if you wait another twenty-nine years and invite me again, I may not be able to make
it—we were in the midst of the eradication of tuberculosis, not only in this State but throughout the nation. The program was prosecuted at great expense and with great success.

In 1929 I went out of office as Director of Agriculture of Illinois, and I left behind me a recommendation that an immediate program should be inaugurated for the eradication of what had then become known as Bang’s disease. I came back in office in January 1953, and I found that that program had been started. We then had an infection in our State of between seven and eight percent on blood tests, which I am happy to report has been reduced to one percent. We are now engaged in this brucellosis program, which we are rapidly bringing to a successful conclusion, as we did the tuberculosis program.

But don’t think our work is done. In livestock disease control, our work is never done. We are well aware that other activities should be engaged in at this time. If I were to go out of office today, which I don’t expect to do, I would leave recommendations that immediate programs be started for the eradication of disease in Illinois and in America.

While I respect to the utmost the work that has been done in the past, and the men who built the foundation of which I spoke, in my recent years of experience I have earned the respect of the men who are doing the job now, the men who are working on the foundation created back in those early days, for I have had that rare opportunity of being honored twice. I was in livestock disease control work back in the 1920’s, and I am back in it now. I only hope that we are as successful in this period as we were then. I think and believe we will be, for I believe that the men who are at the helm in the United States Department of Agriculture and in the several states and the livestock sanitary officials in all the states are proving themselves equal to the occasion.

As we eradicate brucellosis from our nation, we go on into other fields. There is no question about the work that must be done. There is no question about the standing of the veterinary profession in America today. I have seen this profession come up from a position of little standing in our country, until today the researcher in disease control, with all due respect to the medical doctor, the chemist, the microscopist and all the rest—I daresay that the veterinary profession today in research is doing as much as or more for the economy and the health of America and the world as any other group.

There has been a great challenge in the past. That challenge has been met on every occasion. There never was a greater challenge than there is today, and there never was as well qualified a profession to meet that challenge.

I can do no more than emphasize your responsibility to humanity. Don’t ever think lightly of your profession. Don’t ever think lightly of the job that is yours to perform, not only for the livestock industry but for the civilization in which we live.

You are a necessary part and one of the important cogs in the wheel which we need and must have so that we can progress safely and so we can protect the interests of mankind. Your part is not small.

You have this great foundation upon which to proceed to build. You have the
experience of many years. You have officials in Washington and in the several states who are qualified and proven. The future is bright, and the challenge is great.

We have problems before us today that we never dreamed of a few years ago. Just before I left office I received a letter from one of the State institutions in Illinois, and I use this purely as an example. I have known that institution and the farm that exists there, of several hundred acres, for a great many years. I remember when they produced 40 to 50 bushels of corn to the acre on that farm, and they thought well of their crop. In the letter I received yesterday they reported that their average production for the several hundred acres making up that institutional farm was 133+ bushels to the acre.

We have increased production from the soil; we have increased production of meat through our feeding practices; we have accomplished all of these things, but in each instance there comes additional problems and additional challenges. As we fertilize the soil to bring up its production and multiply the bushels per acre, we change the components of the plant. As we change the components of the plant, we know but little of the effect of that plant life upon the animal and human family.

A great deal of research is needed to catch up, if you please, with our production methods in this country, to say nothing of the challenge that disease and new diseases are constantly placing before us.

I mention these matters merely to try, in my feeble way, to impress upon you the importance of not only your profession but your organization, which has stood for all these years as an efficient and a valuable contribution to livestock disease control and the livestock industry and the humanity of our country as well.

To say that you are welcome in Illinois is putting it very mildly. A group of this kind should be welcome anywhere in America. You are welcome in Illinois, and I speak on behalf of the governor of our State. I speak for myself as Director of Agriculture; and if you please, I speak for the citizenry of the entire State.

You are not only welcome here, but I say with all reverence, may God bless you in your endeavors in the future as He has in the past. Thank you.
RESPONSE TO WELCOME

H. U. GARRETT

Des Moines, Iowa

Mr. President, Director Stanard, Ladies and Gentlemen:

On behalf of the United States Livestock Sanitary Association, Mr. Director, I wish to thank you for your fine address of welcome. It is greatly appreciated. You and I have something in common. I, too, am 64 years of age. I have noticed that several times within the last few years. [Laughter]

It is quite fitting that we come back to Chicago for our meeting. Again history has repeated itself. In 1899 we met for the first time as a Livestock Sanitary Association in Chicago. We put the Association on wheels then, and moved about the country, and did not return until 1909, ten years later. We met in this city every year for forty-two years, and then again we put the Association on wheels and started to travel around the country.

We went first to Denver, Colorado. I think the idea was to see how the hearts of us older fellows could stand it. We got by. Then we settled in Columbus, Ohio for the next meeting, and a wonderful meeting it was. From there we traveled to Phoenix, Arizona. Some parts of that State, you know, are rather dry. I heard a fellow say out there that it was so dry that their cows gave powdered milk, and they milked them with a vacuum cleaner.

From there we went to Kansas City, and the following year we went to Louis-ville to test the resistance of some of us older fellows out at the race track. Some of us didn't play—we just watched; others did quite well, and others—well, by now they are probably broke.

We went from there to Atlantic City. We thought that would stimulate some of us oldtimers. We waited with our meeting that year until the season had closed, and about all we saw was the ocean.

Then we went to the Midwest, and met in Omaha. Last year we met in New Orleans. We tested some of the Southern hospitality, and found it fine indeed.

Now we have returned to Chicago. It is quite fitting, and for some reason I feel closer to the livestock industry when we meet here, with the International Live-stock Exposition being held at the same time. When we visit that Exposition we are reminded of how far the livestock industry has come, from way back when we had livestock that didn't amount to very much. We are producing so much more today than we did in the early days. We have produced enough to give us a surplus.

I have never been convinced that we have a surplus. Perhaps we have not had an equal distribution. I think it is necessary that we approach that distribution from a different angle than we have in the past. Perhaps if some of the hungry people in the world were fed with food instead of given dollars, we and the world both would be better off. We would have less of a surplus, and I am quite sure there would be less hungry people going to bed throughout the world.
We have come a long way in disease control. We have discovered a lot of answers that we didn’t have in the past. We have new treatments. When I began practice in 1914 there were a number of things that we had to treat by symptoms. We knew little about them. We had very little sound treatment for many cases. Today that has been changed. Many diseases we had then have been entirely eliminated. We have almost eliminated glanders in the horse. When I was a young chap, just out of school, there was considerable glanders throughout the country. I remember that in the larger cities the drinking troughs were polluted by infected animals, until those drinking troughs were eliminated.

Today while we have eliminated a lot of disease, our challenge is greater than ever before. We have more people to feed. We must look to the production of food in our country to be distributed over the entire world.

Recently I read a report of the activities of the Meat Inspection Branch, and I was surprised at what I found. Under federal inspection, 100 million animals are slaughtered annually. Of that number, almost 4,000 are condemned animals; 300,000 carcasses are condemned on post mortem; 2½ million parts of carcasses are condemned. That all reaches back to the farm. We need to have a better understanding of disease control on the farm, better sanitary methods, and closer cooperation between the livestock industry and the veterinarian. Until we have that, we are not going to be able to produce the extra supply of food that we need for the future.

A few years ago I read that the Bureau of the Census in Washington estimated that our population in 1975 would reach 200 million people. I am inclined to go along with that estimate. With the activity we see by young people today, I think they will beat that figure.

Mr. Stanard, again it is my very great pleasure to thank you for your very kind words of welcome. [Applause]
PRESIDENT'S ADDRESS

A. L. BRUECKNER

College Park, Maryland

Members of the United States Livestock Sanitary Association, distinguished guests, ladies and gentlemen, it is a distinct honor to have this opportunity to address you as President. The year of my office has been a quiet one in that nothing has occurred which necessitated an emergency meeting of the Executive Committee. Our efficient Secretary-Treasurer, Ralph Hendershott, has kept routine matters in order and on occasion has burst forth with something of general interest.

The program to be presented to this Sixtieth Annual Meeting of the United States Livestock Sanitary Association will encompass almost all phases of disease control and eradication, with the inclusion of some research subjects and some other material which may appear somewhat foreign to the responsibilities of disease control officials. This leaves little material from which the President may develop a dissertation worthy of presentation to such a gathering as is assembled here. Under these conditions, it becomes necessary, and I trust not inappropriate, that I refer in a general way to some of the important developments in certain fields, the details of which will be given in committee reports and in the associated presentations.

Tuberculosis still presents serious problems after 39 years of effort toward eradication. The disease is not as widespread in the cattle population as in the early years, but in isolated cases it presents a problem of serious proportions to the individual herd owners of infected herds. Tuberculosis, as we now know it, is capable of spreading rapidly and extensively in infected herds and to many other herds through transfer of animals. The percentage of reactors and the total number of reactors for 1956 increased over the previous fiscal year. Efforts of control agencies to identify the disease in straight kill cattle and to trace these cases to the herd of origin are paying dividends in locating foci of infection. Programs for the eradication of tuberculosis in poultry, swine, and other mammalian species should be intensified. Eradication is possible, and no one should be satisfied until this is accomplished.

Bovine brucellosis is being controlled and eradicated at a remarkable rate. The number of herds under supervision and the numbers of modified certified brucellosis-free counties and states are being multiplied rapidly. The percentage of infected herds and cattle being disclosed is decreasing each year and the number of reactors held is also declining. Retention of reactors, certainly not desirable, does not pose the same problem at this stage of the program as it did earlier, because of the increased percentage of calf vaccinated animals present in herds. Vaccination deserves the renewed support and fullest confidence of everyone concerned, because it offers protection to the individual owner and a relatively cheap means of accomplishing control and eradication, from the state and federal viewpoint. Liberalization of restrictions on vaccinated animals must keep pace with information from research studies, in order that we promote, rather than restrict, the use of the procedure.
PRESIDENT'S ADDRESS

Swine brucellosis control and eradication programs still lag in virtually all states. No one denies that reports of medical authorities point to swine brucellosis as the cause of disease in humans to a much greater extent than to the disease in cattle. The swine producers of my limited acquaintance do not understand why the control and eradication program at the federal and state levels does not apply to them on the same basic principles as are followed with the cattle owner. They feel that they have a right to receive free testing and indemnities. There is no doubt in my mind that, when a program based on these principles is presented to them, they will enter into it readily.

Brucellosis in goats apparently is not an important problem nationally at the present time. It is possible that it can prove more of a danger to human health through milk than in the case of the bovine disease, because of less rigid control of health agencies. Intensification of efforts at this stage can pay large dividends in the future.

There are many other diseases on which this address could elaborate. Mere mention at this time of hog cholera, anaplasmosis, mucosal disease complex, rabies, scrapie, Newcastle disease, infectious bronchitis, chronic respiratory disease, and infectious synovitis must suffice. Discussions of these and others appear in the program.

The Agricultural Research Service of the United States Department of Agriculture, with its numerous branches, has stimulated programs into action on an accelerated scale through requests for more federal funds and personnel and the liberalization of some federal requirements. It is likely that this was possible, to some extent, because of a fresh outlook on the work within the Department. A committee from the United States Livestock Sanitary Association has met with the Agricultural Research Service in efforts to substantiate requests for funds to carry on programs which seemed essential to progress. This procedure of calling the industry and control officials for help has been used in other lines of endeavor in the Department.

It will be recalled that Dr. T. O. Brandenburg of North Dakota, when he was President of this organization, proposed that the office of the federal representative in the state, known then as the inspector-in-charge, be combined with that of the chief livestock sanitary official of the state and that federal funds be deposited with the state for use on cooperative projects. Funds for research from the Office of Experiment Stations and from the Research Branch are handled in this way. An approach to this setup is now in operation in a number of states where there is one cooperative position, a combination of the veterinarian-in-charge and the state sanitary official. The future will tell whether or not this arrangement serves the purposes of the Federal Government and the state to the best advantage.

Federal funds from the Agricultural Research Service for research studies on a cooperative basis, either with individual states or with area groupings, have been very helpful in assisting state research organizations to develop staffs and facilities for the work. In most cases, if not all, the funds expended by the states have been greater than those received from these outside sources. The stimulation of efforts through these grants has paid off to the state in greater measure than to the Federal Government. Strong state-supported organizations for diagnosis and research are absolutely necessary for the livestock and poultry industry and for the
public health, and these should never be allowed to deteriorate because of programs conducted at the federal level.

Cases in point, of course, are the Plum Island Laboratory and the new diagnostic and research laboratories at Ames, Iowa. These are to cost vast sums of money to build and will require large annual sums for maintenance and operation. The staffing must be with individuals of high intellectual stature, coupled with an understanding of the needs of the livestock industry. Research should be conducted to accomplish work which state organizations cannot carry out because of lack of facilities and funds. There is no reason for your President to believe that there can be a monopoly on brains in the federal organizations. It might seem that it has been possible to raise salaries at the federal level to such an extent that there is unfair competition with state organizations and institutions in this regard. We all know that such increases in salary at the state, federal, city, university, or at any other level, may work a hardship for some time but that they eventually cause higher salaries all through the veterinary fields.

Committee reports to the United States Livestock Sanitary Association, when they refer to disease control programs which are conducted on a cooperative basis, have been adopted by the Agricultural Research Service for use at the national level. In some instances in the past, alterations have been made in the text upon adoption by the federal agency. If such changes are made, it is suggested that these be inserted in italics or in quotes with an appropriate footnote as to their source. On occasion, the committee report on tuberculosis or brucellosis contains some change which does not seem applicable or which cannot be made effective at once. This situation may arise because the committee at its hearings, the Association at its regular session, and the Executive Committee in meeting do not realize that the change needs further consideration. Your President thinks that reports of committees which contain recommendations for action, unless of an emergency nature, be held over for a year before being acted upon. The specific committee and the Executive Committee should be able to make the decision as to what needs action at once and what should be held for study.

The final draft of the federal brucellosis regulations for interstate movement of animals should have been received by all chief Livestock Sanitary officials. According to schedule, these regulations go into effect January 1, 1957. They cannot be entirely satisfactory to everyone concerned. There are sections which will not please the Eastern Seaboard, and there are sections which will not please the West. They represent a compromise of interests. The most important consideration in the whole matter is whether or not trucks in interstate livestock business will be forcefully subjected to these regulations, as are railroads. If so, and if federal, as well as state employees, have authority to stop trucks for checking purposes, some of our troubles will be taken care of. As more and more counties and states control brucellosis through better intrastate regulations, including livestock auction markets and dealers, the chances of importing infected animals become lessened.

Your President has been concerned for several years by certain trends in the relationship of the federal disease control agencies to the state agencies. With the more liberal thinking in the Agriculture Research Service about disease control work has come a tendency to expand the responsibilities of the veterinarian in
charge in the cooperating states, to the extent that diseases not covered by a memorandum of understanding between the cooperators come within the scope of his reports. It is true that the Agricultural Research Service was requested to ask for funds for personnel and facilities for preparing reports on morbidity and mortality relating to livestock. I believe that this has been accomplished and that a start has been made.

It now seems to me that the United States Livestock Sanitary Association should have taken over this job by appropriating more funds for the office of the Secretary-Treasurer. The organization has a very definite function to perform in coordinating the work of the chief livestock sanitary officials at the state and territorial level, and this cannot be done except through the services of a full-time Executive Secretary. It is my suggestion that consideration be given at the earliest possible moment to determine the cost of such a move and the means by which this money can be secured.

The relationship of the practicing veterinarians and their clients under the almost universal fee payment system now in use in the cooperative brucellosis programs needs consideration. No one can deny that progress has been rapid beyond the dreams of all concerned and that this would not have been possible on a voluntary testing basis or on a compulsory basis where the owner paid the bill. Nevertheless, it seems to me that a check must be put on the practice of free service, so that the financial responsibility for maintaining the herd free of infection (whether tuberculosis, brucellosis, mastitis, or any other disease) is placed on the owner and not on the state or federal government, so that the practicing veterinarian deals with his clients on a strict business basis. There is a grave danger that some day in the future herd owners may request that they be relieved of the cost of control of more and more diseases. Under these conditions the practicing veterinarians will be working for the Federal Government and the states for the bulk of their income. When this happens, private veterinary practice will be little better than it is presently in Europe and Asia.

I do not question the use of funds from any source nor the procedures followed in the case of emergency programs of control and eradication. Where tests and examinations are available for determining freedom from disease, it must be the responsibility of the owner to prove that his livestock is safe, as far as the economy of the livestock industry and the health of the public is concerned. However, the financial burden of disease control and eradication must be lifted from the herd owner and the tax payer by freeing the country of the causative agent, whether it be that of tuberculosis, of brucellosis, or of any other disease of public health significance. The responsibility for completing the job rests on the chief livestock sanitary officials and the Agricultural Research Service for guidance and assistance.

As President, I wish to take this opportunity to thank in advance all chairmen and members of committees who have made the program possible. Likewise, I wish to express my appreciation to the membership for having had the honor to have acted as your President.
PRESENTATION OF KEY TO PRESIDENT BRUECKNER

R. A. HENDERSHOTT

Trenton, New Jersey

Some years ago we inaugurated in this Association a program of honoring our Past Presidents with some slight token of our appreciation of the work they did during their year in office on behalf of the members of this Association.

There have been many good men precede our currently retiring President. I have had the pleasure and honor of working with quite a few of the men of this Association as your Secretary during their terms of office, and we have always had splendid cooperation from our Presidents. This year has been no exception. When we elect these men to high office in our Association, we know and expect they will come through. Certainly we have never been disappointed.

Dr. Brueckner has worked very hard and earnestly in making committee assignments and assisting the Secretary in arriving at decisions relative to how matters should be handled when they have come before us for decision. In every way he has certainly carried on the high traditions of this office of President of the United States Livestock Sanitary Association.

It is with a great deal of personal pleasure, and I know you join with me, that I present to him this Key of the Association, for the work he has done as President during the year 1955–1956. I know he will wear this with great pride, as I would were I privileged to have one.

Dr. Brueckner, may I present this Key to you. [Applause]

PRESIDENT BRUECKNER: I will certainly have a great many fond memories of the year that I have spent as President of this organization. This will be one of my most prized mementoes. Thank you very much.
4/29/55.


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Lib. E. B. O. T Mail Room
Mr. President and Members of the United States Livestock Sanitary Association:

The past year is up to par in regard to the activities of the Secretary's office. The memberships of the Association, however, are down. For the last decade we have been endeavoring to build up the membership of this Association, and we never quite reach the goal we set for ourselves.

We hit the peak in 1955 with 1,080 paid memberships. This year we dropped to 1,018. That does not seem like too great a drop, but any move backward is always a very serious matter as far as the office of the Secretary of the Association is concerned.

Repeatedly I have asked you men to make an effort to sell at least one membership to someone in your area. In order to encourage and remind you of this, you will notice that when bills are sent out at the beginning of the fiscal year, or at the time of our meeting, they include a dues notice and an application of membership card. Quite a few will readdress that card to me and put their name and address on it, and laboriously include their zone number, and so on, all of which I have on an addressograph plate.

The idea is that you might be stimulated to encourage someone else to put his name on the application card so that our membership might increase. It is very easy to judge how rapidly we would increase our membership if each of you will put on a one-man drive to obtain one new member each year.

This Association and the work it is doing should be better known, and one way for it to become better known, of course, is through an increase in individual memberships.

In this connection, we have had an example in the American Veterinary Medical Association, where some ten years ago, in order to increase interest and members in that Association, they developed what is known as junior memberships, which are open to veterinary students in our veterinary colleges.

I don't know exactly how successful that has been in building their membership; but judging from the number of members in the AVMA, I would say it has met with a great measure of success. How often they are able to get repeat memberships out of that group, I don't know; but in any drive for membership you will pick up perhaps 200 or 300, and several hundred of the group will drop out during the next year. So, we have a constant battle on our hands to keep our membership above the 1,000 mark.

I am sure all of you are interested in the Association expanding its membership, and a little effort on your part, I am quite sure, will help us obtain our goal. We should have in this Association no fewer than 10,000 members.

I would suggest that it might be well to give consideration to having an amendment to our Constitution and Bylaws, whereby we too might enjoy the
benefit of junior memberships among our veterinary students, and also among the students in our agricultural schools. I have even thought of the possibility of going down in to the juvenile area and inviting 4-H Club, Future Farmers of America and vocational-agricultural students to take out junior memberships at a reduced rate in this Association. We need more farm people in the Association who are raising cattle and other forms of livestock.

I would like to ask all of you to give consideration to the possibility of having such junior memberships. I am sure they would be beneficial in building our membership and, like some old organizations in this world, we find that if we start with the youngsters, and train them, usually we have them pretty well trained by the time they become adults, and it is almost second nature for them to pay their dues and think nothing much about it.

Another item that has been of interest to me, and on which I wrote the membership earlier in the year, is the name of this august Association. During the first years of the Association's existence we were known as an Interstate Association of State Livestock Sanitary Boards, and we carried that designation from 1897 to 1910, at which time the name was changed to “United States Livestock Sanitary Association”.

You will remember that I sent a questionnaire to our entire membership. The response to that questionnaire was exceedingly good. We received more than 70 per cent returns. I can tell you very much easier the number who resisted a change in name of the Association, namely, something under twelve members. Most of the twelve complained about the choice of the proposed name, which I had thrown out simply as a feeler, namely, “The American Animal Health Association”.

I didn't go into a great deal of detail explaining why I had suggested “American Animal Health Association” as the new name, but our Association is no longer a United States Livestock Sanitary Association, if we are to judge by the broad field of membership in other countries. We have and always have had quite a few Canadian members in this Association, as well as some members in Mexico. If you will look in the back of the proceedings book you will note that we have members practically all over the world.

It occurred to me that the name, “American Association,” would have the connotation of taking in the entire Americas, and I was even foolish enough to think that some day, perhaps not in my lifetime, we might even encourage Central and South America to become affiliated with us and maintain memberships.

If Bob Smith is alive at that time, it would be a chance for us to go down to Rio de Janeiro and Buenos Aires for a week. [Laughter] I think Bob would vote for that.

The name proposed was “The American Animal Health Association.” As far as our dealings with livestock are concerned, we take in all forms of livestock, and just last evening a new committee was proposed, which would deal with the health of laboratory animals. I am quite sure that our Vice President will have something to say about that, possibly in the Executive Committee.

The word “Health” always is a good word to tie in with a disease control program, both from a political and a general standpoint. When we talk about
"animal health" it really means something, at least to me. When you talk about "sanitation" I am rather inclined, in our area, to think of white wings or fellows that follow a business in the rural areas where we don't have good sanitary facilities. I don't quite like that connotation. I think "Health" would be much more appropriate for our Association, since in all of our activities we are dealing with the health of animals.

I am quite sure that when we go into legislative halls and ask for appropriations, if we were known as an animal health association or an animal health department or division of a state government, a great deal more attention will be given to our requests.

That isn't to say that we haven't been fairly dealt with by state and federal governments, but I do think it would make it a little easier for our legislatures to justify in their minds the appropriations they make in the interest of improving health. We do know that our state departments of health have little difficulty in getting substantial appropriations for their work.

So, I think there are many reasons why "American Animal Health Association" might be a good name for this Association.

One of the objections to such a name is that the initials would read "AAHA" and there is an American Animal Hospital Association which has the same initials and which has to do entirely with small animals and pets. Some thought that might make for confusion. I am not too particularly concerned about what little bit of confusion might result.

I will ask that you give serious consideration to these suggestions, because I really think they are worthwhile. We could benefit from a change in the name of this Association, and I know from the standpoint of the office of the Secretary that when I am called upon to describe what our duties and activities are, and what we stand for, sometimes it would be a little easier if I could tell people we stand for the improvement of the health of the livestock of the nation rather than that we are trying to sanitize everything in Christendom. So much for that.

I might report also that we have had a plethora of meetings called by the Agricultural Research Service, which have taken us from Washington on numerous occasions to Kansas City and St. Louis, and pretty much over the country, in the interest of promoting or making a study of livestock diseases in the various areas, and promoting some activity on the part of industry in those particular areas.

In addition to that, we have had two meetings at which our representative of the Association had to make a trip to Chicago to attend Livestock Conservation, Incorporated, and a third meeting at which we were represented by our good friend Dr. Pat Hutchings, from Lafayette, Indiana. All of these add to the cost of operating the Association.

As usual it has been a pleasure to serve you and to work with the officers and committees during the year. I wish to thank all who have cooperated to make this meeting and program possible. Thank you.
REPORT OF THE AUDITING COMMITTEE

K. J. Peterson, Salem, Oregon

T. O. Brandenburg, Bismark, North Dakota

J. W. Green, Indianapolis, Indiana

Gentlemen: Your Auditing Committee has examined the books and records of the Secretary-Treasurer and found them to be correct and in good order. The Committee recommends that the report of the Treasurer be accepted.
MEMORIAL SERVICE

M. N. REIMENSCHEIDER

Oklahoma City, Oklahoma

President Brueckner, members of the Association, ladies and distinguished guests:
To the best of my information, the following members have been called to the Great Beyond since our last meeting:

J. LEONARD AXBY

J. Leonard Axby (CVC '03), 79, Indianapolis, Ind., died on April 17, 1956, after a prolonged illness.
Born in Guilford, Ind., July 28, 1876, Dr. Axby was active and prominent in both civic and professional affairs in his state, and nationally, for more than a half-century. He attended National Normal University of Lebanon, Ohio, and taught school in his native Dearborn County before entering Chicago Veterinary College. Following graduation, he was a member of the faculty of Cincinnati Veterinary College from 1903 to 1919. He also practiced in Lawrenceburg for many years, was mayor there from 1910 to 1914, and served in the Indiana legislature from 1917 to 1919.
From 1933 to 1945, Dr. Axby was state veterinarian, the longest continuous service in the history of the post.
Dr. Axby was a past-president of this organization and during his association with us he delivered the Memorial Address on many occasions.

WARREN B. EARL

Warren B. Earl, aged 71, director of the Division of Animal Industry for the state board of stock commissioners of Nevada passed away, August 18, 1956.
Born in New York on April 9, 1885, he received his early education in his native state, and was graduated as a doctor of veterinary medicine from George Washington University in 1912.
He entered the service of the bureau of animal industry of the United States Department of Agriculture in 1914, and was assigned to stations in various parts of the United States. He resigned from the federal department in 1918 to become field veterinarian under the state board of stock commissioners of Nevada, cooperating with the University of Nevada. He held this position until 1931, when he was named director of the division of animal industry.
He was a member of state and national scientific organizations.

ADOLPH EICHHORN

Adolph Eichhorn, aged 82, of Pearl River, N. Y. died in Miami Beach, Fla., on January 28, 1956, after a brief illness. He was a graduate of the Royal Hungarian Veterinary College, Budapest, class of 1895, and of the New York-American Veterinary College, class of 1900. In 1901, he entered the service of the United States Bu-
MEMORIAL SERVICE

reau of Animal Industry. He was the chief of the Pathological Division when he re-
signed, in 1916, to enter commercial work. He returned to the B.A.I. in 1938, as
director of the Animal Disease Station at Beltsville, Md., where he remained until
1943.

CHARLES E. FIDLER

Charles E. Fidler (CVC '05), 73, Lewistown, Ill., passed away June 6, 1956.
Doctor Fidler was county veterinarian for Kankakee County for several years
and had also served as superintendent of the Division of Animal industry for the
state department of agriculture during the Dwight H. Green administration.

JOHN L. KIXMILLER

John L. Kixmiller (IND '15), 66, Indianapolis, Ind., died July 12, 1956,
after
an illness of several months. Widely known as an authority on swine diseases and
a veterinary consultant for Pitman-More Company for many years, he had also
served his profession notably by his active interest in local, state, and national
association work.

John Kixmiller was one of the most widely known and highly respected
veterinarians in the profession and in the circles which he served so faithfully
and quietly. He will be sorely missed by his countless friends.

ASHE LOCKHART

Ashe Lockhart, 66, former president of Ashe Lockhart Laboratories, died at
his Kansas City home, January 11, 1956.

Dr. Lockhart, a native of North Carolina, was graduate of Virginia Polytechnic
Institute in 1911, and the Kansas City Veterinary College in 1915. Formerly
associated with the Kinsley Laboratories and a Veterinary Corps Lieutenant in
World War I, he opened his own production laboratories in 1926.

A sincere devotee to the advancement of veterinary medicine and the interest
of practitioners, Ashe Lockhart will long remain in the memory of veterinarians
throughout America.

WILLIAM E. LOGAN

William E. Logan (COL '21), 58, Fort Worth, Texas, passed away on July 21,
1956. Born on July 1, 1898, at Cheyenne, Wyo., Dr. Logan had been with the
Bureau of Animal Industry for many years, having served as federal inspector-
in-charge in Kansas, Pennsylvania, and Texas, the latter since 1953. Dr. Logan
was widely known and highly respected by the profession. He will be sorely missed
by his many friends.

DAVID FRANKLIN LUCKEY

David Franklin Luckey died at his home in Tarkio, Mo., on May 3, 1956. He
was a graduate of the Ontario Veterinary College, class of 1898.

For about 21 years (1900–1913, 1914–1922), Dr. Luckey served as State Veter-
inarian of Missouri. He served as president of this organization for the year 1906–
1907. He was awarded the Twelfth International Veterinary Congress Prize at the 1944 meeting of the A.V.M.A. in recognition of his pioneer work in connection with tuberculosis eradication.

Dr. Luckey deserves a great share of the credit for developing the intradermal test for tuberculosis in cattle. Year after year, he reported on this work at meetings. Not until the late Dr. C. M. Haring and his associates in California corroborated Dr. Luckey's work did this test receive the serious attention of the United States Bureau of Animal Industry.

Having been trained for the teaching profession and having served as Superintendent of Schools at Aurora, Mo. (1891–1893), it was natural that Dr. Luckey's interests would extend beyond the realm of veterinary medicine. He had lived in semi-retirement during recent years but he was actively interested in a study of poliomyelitis.

R. S. ROBINSON

R. S. Robinson passed away July, 1956. Age 65, after a lingering illness complicated by Diabetes and the results of an automobile accident which occurred several years ago.

Dr. Robinson practiced at Madison, South Dakota before becoming State Veterinarian for a short period in 1936. He was again appointed in 1938 and held the position until July 1, 1954. During the time between his retirement and his death he worked intermittently as an inspector for the Livestock Sanitary Board.

LEUNIS VAN ES

Leunis Van Es (ONT '93), 87, Lincoln, Neb., died Aug. 27, 1956. Born in Melissant, Holland, Oct. 3, 1868, Dr. Van Es received his early education in that country.

The life story of Dr. Van Es includes his immigration to Nebraska in 1889; graduation (V.S.) from the Ontario Veterinary College in 1893; practicing in Nebraska and in Alabama; earning his M. D. degree at the University of Alabama in 1898; teaching histology and bacteriology at the latter school for five years; heading the Department of Veterinary Science at North Dakota Agricultural College and serving as state Veterinarian for 15 years; then, from 1918 to 1946, heading the Department of Animal Pathology and Hygiene at the University of Nebraska.

Dr. Van Es was a member of many professional and scientific societies, including this organization of which he was president in 1927. Among the honors bestowed upon him were the honorary degree of Doctor of Science by the University of Pennsylvania (1935); honorary degree of Doctor of Agriculture by the North Dakota Agricultural College, which also dedicated its newly remodeled laboratory in his honor in 1952 and erected a bronze plaque commemorating his work at that institution; and the Twelfth International Veterinary Congress Prize, awarded him by the American Veterinary Medical Association in 1953.

CLIFTON D. LOWE

Clifton D. Lowe (OSU '10), 72, Washington, D. C., died Dec. 23, 1955. Nationally known as a livestock authority, Dr. Lowe had served as a judge in many
purebred and market livestock events throughout the country. For many years he was a contributor to the leading agricultural and livestock publications.

After receiving his D.V.M. degree, Dr. Lowe served as instructor in animal husbandry at the Pennsylvania State College. He then was appointed veterinary inspector for the Bureau of Animal Industry, United States Department of Agriculture. He was for 28 years the joint representative of the Bureau of Animal Industry and Extension Service in coordinating and fostering educational programs and activities in livestock production for the Agriculture Department in cooperation with land-grant colleges. A month before he retired, in 1951, Dr. Lowe was awarded the Superior Service Award of the United States Department of Agriculture for his work with the Department.

Dr. C. D. Lowe was an active member of many national organizations.

May I respectfully request all present to rise and remain standing for a short period of silent prayer for the peaceful repose of the souls of these departed colleagues.

Thank you ladies and gentlemen, for your respectful participation. Your speaker feels very humble and inadequate in trying to properly memorialize these Great Men. Their many contributions to their chosen fields will certainly be remembered long after anything that may be said here today. Their contributions to the fields of medicine, veterinary medicine, regulatory services, and all associated fields, have been, and shall continue to be, of untold value to vast segments of people in our Great Land. Certainly the world will be a better place in which to live as a result of their having passed this way.

No words can adequately express the keen sense of personal loss all of us feel on this occasion. Their passing has left vacancies which will be very difficult for us to adequately fill. We need but to remember the contributions made by each of these outstanding men, and to strive to carry on in their place to the end that the lessons they so ably taught shall continue to bear fruit.

Thus we memorialize these departed members, resolving to emulate the goodness and grandeur so well exemplified by their lives.
PRELIMINARY REPORT OF EASTERN EQUINE ENCEPHALOMYELITIS VACCINATION STUDIES IN NEW JERSEY PHEASANTS DURING THE 1956 EPIZOOTIC

Oscar Sussman, D.V.M., M.P.H.,* Daniel Cohen, D.V.M.† and Jean H. Gerende, B.S., M.S.P.H.‡

INTRODUCTION

From the time that Eastern Equine Encephalomyelitis was first reported in ring-necked pheasants by Tyzzer, Sellards, and Bennett in Connecticut in 1938, (1) Beaudette et al. have described 26 outbreaks occurring in New Jersey flocks of commercially raised pheasants from 1939 to 1953 (2-8). Doctor Beaudette concluded, "...EEE occurs with sufficient frequency to pose a serious economic problem to breeders of ring-necked pheasants in New Jersey and perhaps in other highly endemic areas. For this reason and because of its public health importance, further effort should be made to elucidate the epidemiology of EEE and to devise practical methods for its control." (8)

The work discussed in this preliminary report is an attempt, under controlled field conditions, to test the practical use of a formalized chick embryo vaccine in the control of EEE in commercially raised pheasants in a highly endemic area. It was performed in the midst of the 1956 epizootic in New Jersey which affected 48 horses and 19 pheasant flocks from August to November of that year.

HISTORY

On August 27, a farm at Deans, New Jersey belonging to a commercial breeder having approximately 5,000 pheasants was visited. The farm had lost more than 1500 birds and Eastern Equine Encephalomyelitis had been diagnosed by virus isolation at Rutgers University. The disease first appeared in a pen (1) containing 1800 birds on August 16. By September 4, the epidemic in this pen had subsided with a loss of 78.9 percent. The second pen to be affected (2) contained about 1800 birds which became ill on or about August 22. This pen adjoined 1 for about 150 feet. Birds in pens 1 and 2 were about the same age and were 11-14 weeks old when affected. As of August 27, the farmer had already lost 238 birds in pen 2. On August 28 the pen was divided into two sections. Pen 2B was left unvaccinated and 48.5 percent of the pheasants in pen 2A were vaccinated on August 29 and 30.

A third pen (3) was made up of birds four to five weeks younger than those in the first two pens and its fence adjoined pens 1 and 2 at various points. On...
August 27 there were no birds affected in this pen of 860 pheasants. On that date, 530 or 61 percent of this group were vaccinated. Several weeks later, 402 birds (60.4 percent of which had been vaccinated) were selected by the farmer and moved to another pen. Shortly thereafter, on September 21, an outbreak occurred in the 458 birds which remained in pen #3. These birds were about 12 weeks of age and 61.1 percent of them had been vaccinated.

In two other areas on this premise, there were 500 birds raised on wire which had never touched the ground and a pen of breeders retained from the previous year. These pens were 250 and 33 feet distant, respectively, from pens 1, 2, and 3. Neither of these two groups of birds became clinically affected. Of the 500 birds on wire, 400 were vaccinated and 100 were left as controls.

On September 19, the disease broke out at a Jamesburg farm five miles distant which had obtained its birds as chicks from the Deans farm. A statistical analysis of the Jamesburg outbreak indicated that it was comparable to the Deans outbreak in mortality rate. The results of investigations in this pen are included in the vaccine studies undertaken at Deans.

**PROCEDURE**

The vaccine was tested under two circumstances:

1. In an affected pen—one which had already undergone seven days of an epidemic (pen 2 and Jamesburg).
2. In a non-infected pen—among apparently healthy birds which several weeks later were exposed to a severe epizootic (pen 3).

The procedure employed was as follows: The management of the pens and disposal of dead birds were undertaken. Controls were maintained in every pen used. Dead birds were picked up daily and the pens in which they died, their vaccination status and the date of death were noted. Heads were frozen and saved for virus isolation and birds were opened and checked for post-mortem lesions. Sera were taken from representative birds in all pens at varying intervals.

The birds were vaccinated with a repeating type vaccinating syringe.* The same needle was used for fifty to seventy-five birds. A 0.2 ml dose of undiluted formalized chick embryo bivalent vaccine† was inoculated intramuscularly into the pectoral region. In the instance of the particular vaccine employed, the amount used for a single injection in 100 pheasants would have provided enough vaccine to immunize five horses with a double injection applied intradermally. In each pen, fifteen birds were vaccinated and stained with a green dye on the medial surface of the left wing. Ten to fifteen birds, depending on the proportion of non-vaccinates desired, were then stained with a red stain on the medial surface of the left wing and allowed to go through without vaccination. The non-vaccinates were allowed to run with the vaccinates in the same area.

* Vipol Intramuscular Vaccinator produced by Vineland Poultry Laboratories, Vineland, N. J.
† This vaccine was generously provided by Pitman-Moore Laboratories, Indianapolis, Indiana.
EEE VACCINATION IN PHEASANTS

TABLE 1
Mortality in Epidemic Pens Vaccinated Seven Days After Onset of EEE

<table>
<thead>
<tr>
<th>Pen</th>
<th>No. of Birds in Flock</th>
<th>No. of Birds Vaccinated</th>
<th>Percent of Birds Vaccinated</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deans 2B</td>
<td>480</td>
<td>0</td>
<td>0.0</td>
<td>71.1</td>
</tr>
<tr>
<td>Deans 2A</td>
<td>1113</td>
<td>540</td>
<td>48.5</td>
<td>44.4</td>
</tr>
<tr>
<td>Jamesburg</td>
<td>524</td>
<td>524</td>
<td>100.0</td>
<td>29.8</td>
</tr>
</tbody>
</table>

RESULTS

VACCINATION IN AN AFFECTED PEN

The disease had already run its course in pen #1. In pen #2, clinical infection had been going on for seven days. The disease usually runs a two to three week course. This pen (#2) was divided into Section A containing 1113 birds and Section B containing 450 birds. 540 or 48.5 percent of the birds in Section A and none of the birds in Section B were vaccinated. At Jamesburg, seven days after the outbreak occurred, 100 percent of the 524 birds remaining were vaccinated. There were thus three groups representing three levels of vaccination in comparably affected pens seven days after infection had begun; i.e., 0, 48.5 percent and 100 percent.

The results in these three groups are summarized in Table 1.

There is an inverse relationship between the percent of mortality of the three groups as opposed to the percent of vaccination. It is interesting to note that when the mortality of the first seven days was proportionately allotted to pen 2B where no vaccination was employed, the total mortality rose from 71.1 percent to 74.9 percent which is statistically comparable to the 78.9 percent noted in pen #1. Within pen 2A, where 48.5 percent of the birds were vaccinated, the difference in the mortality rates between the vaccinates and the non-vaccinates is also significant. The non-vaccinates had a mortality rate of 61.6 percent as compared to a 26.1 percent rate for the vaccinates. A significant difference in these rates was first noted seven days after vaccine was employed.

VACCINATION IN A NON-INFECTED PEN

The second part of the experiment involved pen #3. This pen containing 860 birds showed no signs of infection when vaccinated. As indicated earlier, 530 or 61 percent of this group were vaccinated. Several weeks later, the farmer moved 402 of the most completely feathered, least cannibalized birds from this pen to a clean area where they were debeaked. Two days later, the disease broke out in the birds which remained in pen 3. Of the remaining 458 birds, 61.1 percent had been vaccinated.

The results are summarized in Table 2.

The overall mortality rate in this pen, where vaccine had been administered to 61.1 percent of the flock three weeks prior to the outbreak, was 34.7 percent. This latter figure must be compared with the 74.9 to 78.9 percent mortality which was
TABLE 2

Mortality in Pen #3 Vaccinated 3 Weeks Prior to Onset of EEE

<table>
<thead>
<tr>
<th>Description</th>
<th>Number of Birds</th>
<th>Percent of Flock</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>280</td>
<td>61.1</td>
<td>35</td>
</tr>
<tr>
<td>Non-vaccinates</td>
<td>178</td>
<td>38.9</td>
<td>124</td>
</tr>
<tr>
<td>Total Flock Pen #3</td>
<td>458</td>
<td>100.0</td>
<td>159</td>
</tr>
</tbody>
</table>

to be expected had no vaccine been employed (as in pens 1 and 2B). A significant difference between the vaccinates and the non-vaccinates is the fact that in the case of the non-vaccinates, it was a mortality rate of 69.7 percent of the only 38.9 percent non-vaccinates available; whereas, the 12.5 percent mortality was noted in the 22 percent larger group of birds vaccinated.

DISCUSSION

The results offer an index to the efficacy of a formalized chick embryo equine encephalomyelitis vaccine in commercially raised pheasants in New Jersey. The challenge to which these birds were exposed was apparently of high virulence sufficient to produce a mortality of 74.9-78.9 percent in non-vaccinated pens. The vaccine produced a significant degree of protection when employed in pre-epidemic and epidemic situations.

No attempt was made to evaluate debeaking as a measure of control. It is felt that once a disease enters a pen, cannibalism and feather picking may possibly serve as mechanisms for its spread. In this connection, it should be noted that only three birds died of the 402 birds debeaked and transferred to a clean area from pen 3 two days prior to the outbreak in that pen. It would be difficult to ascertain under the circumstances just how much credit should be given in this instance to debeaking and how much should be given to other factors; i.e., new environment or condition of birds selected.

Wildlife management personnel have questioned the advisability of debeaking late in the season, the time when the disease normally occurs. They believe such birds must be retained beyond the normal release period until their beaks grow out, since the survival ability of debeaked birds in nature is not good. Some other method of supplying protection from this disease, such as vaccination, would be preferred by them.

The commercial breeder who does not intend to release his birds may question whether it is financially worthwhile to vaccinate. The cost for vaccination on a private professional basis is estimated under New Jersey conditions at about 10-15 cents per bird. This will vary somewhat depending on the cost of vaccine used, the number of birds handled, and the ease with which the birds are caught. In New Jersey, birds for release sell for $3.00 to $4.00 each. The disease usually strikes when the birds are fully grown and the owner has already invested his labor and feed in the flock. It, therefore, does not appear too excessive a fee, in a highly endemic
EEE VACCINATION IN PHEASANTS

area, for the insurance the vaccine may provide against losses incurred after a full season's work.

Vaccination, under the conditions and dosage described in these studies, apparently does not supply the complete answer to the problem of EEE in pheasants. Some vaccinated birds did succumb to the disease when the challenge was sufficiently great. However, this preliminary report indicates that epizootics in pheasants can be significantly curbed by proper vaccination and the economic losses and public health hazards of EEE substantially reduced.

ACKNOWLEDGEMENT

Acknowledgement is made for the field assistance rendered to this project by William C. Carter, D.V.M., M.P.H. and Robert Goldsboro, D.V.M., M.P.H. of the New Jersey State Department of Health.

REFERENCES

Gentlemen: Doctors Jas. R. Hay of Ohio, J. L. Stuart of California and myself represented the Association at the 38th Annual Meeting of the Commissioners, Directors and Secretaries of Agriculture held in San Francisco September 14 to 22 last.

We were present at the Animal Industry Committee Meeting which was conducted under the chairmanship of Mr. S. J. Stanard, Director, Illinois Department of Agriculture.

There were eight resolutions developed by this Committee and later adopted by the Convention that are of interest to the United States Livestock Sanitary Association and turned over by me to our Committee on Resolutions. These resolutions dealt with the following topics.

1. Mastitis Research
2. Swine Brucellosis
3. Hog Cholera Suppression
4. Mandatory Cooking of Garbage for Swine Feeding
5. Compulsory Inspection of Poultry
6. Training Courses in Regulatory and Inspection Work
7. Scrapie Eradication
8. State Meat Inspection
UNITED STATES LIVESTOCK SANITARY ASSOCIATION ADVISORY COMMITTEE TO THE AGRICULTURAL RESEARCH SERVICE ON PROGRAM AND BUDGET

Dr. W. L. Bendix, Richmond, Virginia; Dr. A. P. Schneider, Boise, Idaho; Dr. H. U. Garrett, Des Moines, Iowa; Dr. R. A. Hendershott, Trenton, New Jersey; Dr. G. H. Good, Cheyenne, Wyoming

The Advisory Committee held three meetings during the year—two in Washington, and one in San Antonio. These meetings were held with representatives of the Agricultural Research Service and involved discussions regarding this service, program and budget.

Considerable time was spent discussing the Tuberculosis Program. The Committee agreed and will recommend, that the administration of the program involving the accreditation and re-accreditation of counties should be improved. The Committee requested that when states become delinquent in their re-accreditation work and give no evidence of being able or interested in re-accrediting counties, that these counties be dropped from the accredited list, and public notice be made thereof. It was agreed that before such action be taken, that every effort be made by the Agricultural Research Service, working with the veterinarians in charge and the state livestock sanitary official in such states, to improve the situation and bring as many counties up-to-date as possible. There was considerable discussion about the new proposal for the re-accrediting of counties which were approved by the United States Livestock Sanitary Association at its 1955 meeting and which are presently under review under the Animal Disease Eradication Branch. The Committee requested the Branch to circulate this proposal fully throughout the country, and ask for additional comments before adopting this proposal.

The Committee discussed at some length the lack of uniformity in fees being paid practitioners in the Accelerated Brucellosis Program. A request was made that the Agricultural Research Service institute a study of the fees being paid, and review the need for adjustments in some of the states in order to bring the fees more in line with surrounding areas. The Committee feels that as a result of this study it may be possible to reduce the rate of pay in some areas, and that any adjustments deemed proper should be downward, provided, it does not interfere with successfully carrying on this project.

The subject of Scabies in sheep came in for a full discussion which resulted in the Committee requesting the Agricultural Research Service to consider a Federal Regulation on the interstate movement of sheep which would require dipping except from states declared free of Scabies. The Committee requested also, that no official action be taken until an opportunity had been provided to discuss this matter with all of the states concerned.

The Scrapie Program was reviewed briefly and the Committee concurred in a suggestion that a meeting be held which would bring together all of the information available in the United States and Canada about this disease so that consideration could be given to some modification of the present program if it seemed necessary. Three such meetings have been held during the year—one in Washington at which all interested professional personnel from the United States and
Canada were present; one in St. Louis in which industry was invited to participate, and a final meeting in Washington dealing with the research obligations and needs regarding this disease. Your Committee was represented at all of these meetings.

Discussions pertaining to the Import-Export Inspection and Quarantine Service was of great interest to the Committee, particularly the report that there were additional funds available for this work in the 1957 budget. Your Committee has felt all along that in this sphere of operation the Agricultural Research Service was very weak, and had strongly recommended increased funds and increased personnel for this important service.

The Committee was particularly interested in the inspection and licensing of veterinary biologicals. The Committee was seriously concerned about the loss of laboratory facilities resulting from the closing of certain laboratories. In this sphere of operation the Committee feels the Agricultural Research Service is also very weak, and has repeatedly urged strengthening and enlargement of the scope of this activity. It is very gratifying to know that such projects are underway.

In regard to the Meat Inspection Service, the Committee was presented with figures showing how the work-load is constantly increased, while the personnel for this work is constantly decreasing. This is primarily a matter of finances and personnel.

The following formal recommendations were made and passed to representatives of the Agricultural Research Service and the Bureau of the Budget. These recommendations in no sense represent all of the areas in which the Committee feels increased activities should be undertaken but merely those matters considered to be most urgent at this time. The recommendations are as follows:

1. In view of the increasing population in the United States and the increasing per capita consumption of meat and meat food products, we recommend and urge an increase in the annual appropriation for the Meat Inspection Branch of $900,000. This figure is based on actual demand and actual rate of growth in industry requiring this service.

2. We recommend that an additional $200,000 be provided for diagnosis, control and eradication of miscellaneous diseases. The increasing importance to the national economy of such conditions as bluetongue, scrapie, mucosal disease, rhinotracheitis, ornithosis, vesicular stomatitis, and the virus diseases of poultry make this a most important and urgent item.

3. We recommend an additional sum of $100,000 to provide for the control of manufacture, importation, shipment and marketing of viruses, serums, toxins, etc. The fantastic growth in the manufacture and use of animal and poultry biological products makes this item one of paramount importance.

4. In the face of an ever expanding livestock economy in the nation, disease and parasite research are of vital importance. The present research projects in both diseases and parasites reflect about the maximum possible with existing facilities. Research projects totaling an annual expenditure of approximately $300,000 do not reflect a realistic approach to these problems. The need for expanded facilities in research is urgent and vital. We urge that every effort be made to expedite the construction and operation of adequate research facilities with sufficient personnel.
For many years, regulations governing the interstate movement of livestock based on the health status, promulgated by an agency of the Federal Government, have been in effect. These regulations, among others, pertain to the testing of cattle for tuberculosis, dipping of sheep and cattle before interstate shipment to prevent the spread of scabies, and the vaccination of swine to be moved from public stockyards. Practically all states have, from time to time, enacted state regulations with more stringent requirements than are included in the Federal regulations.

During the past year, the Agricultural Research Service had promulgated a Federal regulation which becomes effective on January 1, 1957, regulating the interstate movement of cattle according to their brucellosis health status. This regulation is officially identified as "Title 9, chapter 1, sub-chapter C, Part 78, sub-parts C and D."

Your committee endorses the principles of this Federal regulation and urges state regulatory officials to cooperate in its enforcement. Your committee further urges the Agricultural Research Service to use their full resources and efforts in enforcement measures, and also to use every means at their command to cooperate with State Livestock Sanitary officials in the enforcement of the importation regulations of the several states.

Your committee recommends that this Association evaluate the results of the enforcement during the coming year and at the termination thereof, make recommendations to the Agricultural Research Service for any changes that seem to be indicated.

It is also recommended that all State officials make every reasonable effort to enforce their State regulations, as has been done in the past, and to cooperate with other States by approving only such health certificates as meet the requirements of the State of destination.

**Standard Regulations**

1. Amend Section 1515-CATTLE, the first paragraph, as follows: Delete the word "certificate" following the word "The", and preceding the word "must", all on the third line of said paragraph, and insert in lieu thereof the word "veterinarian". The last sentence of this paragraph as above amended to be printed in bold faced type.

2. Amend Section 1515-CATTLE, by inserting a new paragraph following the phrase "with regard to tuberculosis:" to read as follows:
   A. The intradermal and subcutaneous tests are acceptable if conducted
by an accredited veterinarian approved by the chief livestock sanitary official of the state of origin. The type of test shall be indicated on the health certificate.

Reletter present paragraphs A and B to paragraphs B and C, respectively.

3. Amend sub-section II, paragraph C to read as follows:
   C. They are officially vaccinated when between 4 and 8 months of age, or in the case of beef type calves in range or semi-range areas, between 4 and 12 months of age, by a qualified veterinarian, are under 30 months of age at time of shipment, are individually identified by ear tag or individual tattoo number, and originate from herds not under quarantine for brucellosis. The health certificate shall include the date of vaccination of each individual animal in the shipment and the name of the veterinarian conducting the vaccination, if different from the veterinarian issuing the health certificate.

4. Amend sub-section II, paragraph D, as follows:
   Delete the word “incomplete” in the last line and insert in lieu thereof the word “complete”, and delete the figures in the last line “1:100” and insert in lieu thereof the figures “1:50” or less.

5. Re-number the paragraphs in the “Standard Regulation” in the manner followed in the Report of this committee in 1954 and published in “Circular 1” of that year.

Circular 1

6. Your Committee recommends Circular 1 be revised and re-published at an early date. It is further recommended the “Standard Regulations” adopted by this Association at their 59th Annual Meeting and as published in the Proceedings, pages 40 to 46 inclusive, as amended in this report, be published as a foreword of said Circular. It is also recommended the requirements for each state be shown as compared to the standard regulations. For example, if the state requirements are covered by the Standard Regulations, they should appear under the heading of the state as follows:

   “1516 DOGS. Standard regulation applies,”

or if there is some variation they should appear as follows:

Section 1515 CATTLE.
1. Dairy and Breeding.
   With regard to Tuberculosis:
   “Standard Regulation applies. Also cattle originating in herds in modified accredited tuberculosis-free areas if the entire herd of origin has been tested and found negative within 12 months, may be imported without a further tuberculin test.”

   It is further recommended the incoming committee on Laws and Regulations prepare a questionnaire chart, similar to the chart prepared and published by New York, and other states, for each species of livestock, namely cattle, dogs, horses, sheep and goats, swine, poultry and Psittacine birds, covering state requirements
for importation; said charts to be mailed to each chief State Livestock Sanitary official. When completed, the questionnaire chart shall be returned to the committee on Laws and Regulations for compilation. It is further recommended, the compiled chart to be included in the report of the 1957 Committee on Laws and Regulations.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION
COMMITTEE ON LEGISLATION

W. L. BENDIX, Richmond, Virginia, Chairman; Dr. T. C. GREEN, Charleston, West Virginia; Dr. J. V. SMITH, Hartford, Connecticut; Dr. O. HALL, Ottawa, Ontario, Canada; Dr. R. W. SMITH, Concord, New Hampshire; Dr. H. J. ROLLINS, Raleigh, North Carolina; Dr. H. F. WILKINS, Helena, Montana

There was introduced into the 84th Congress, 2nd Session, the following bills in which the United States Livestock Sanitary Association was interested.

Several bills were introduced extending the Accelerated Brucellosis Program for two additional years, and appropriating additional funds for the fiscal year ending June 30, 1956 for this purpose. Congress finally passed, and the President signed a bill providing $2,000,000 additional for the 1955–56 year, and $20,000,000 for each of the fiscal years ending June 30, 1957, and June 30, 1958. The United States Livestock Sanitary Association indorsed this bill and the committee members in both the House and Senate were so advised.

H. R. 9744. This bill relieves the Secretary of Agriculture of the necessity of notifying common carriers directly or by newspaper publication of the existence of certain contagious diseases in the territories or areas served by such carriers. Publication of such notice in the Federal Registrar by the Secretary, and in such other manner as he may deem appropriate is considered sufficient notice and is so provided in this bill. The United States Livestock Sanitary Association indorsed this legislation, and the committee members in the House were so informed. A companion bill, S-3046, was introduced in the Senate.

Congressional Action: Did not pass.

H. R. 8540 and H. R. 9603. Introduced to require the use of humane methods in the slaughter of livestock and poultry in interstate and foreign commerce. The United States Livestock Sanitary Association took the position of favoring any sound law that would contribute to this end, but these particular bills were not well thought out and that the whole subject needed a good deal more study before the enactment of laws. The committee was notified of the Association's position.

Congressional Action: Killed in Committee.

H. R. 11682, H. R. 11699. Introduced into the Congress to amend organic acts of the Department. During the course of congressional consideration of the supplemental appropriation bill for 1956 several appropriation items were stricken from the bill since they contained some provisions which were legislative in character. The purpose of these bills is to simply avoid similar questions arising in the future. A companion bill was introduced in the Senate as S-3991. The United States Livestock Sanitary Association indicated its approval of these bills to both the House and Senate Committees.


S-3668. This bill was introduced into the Congress to provide further protection against the dissemination of diseases of livestock and poultry. The Secretary of Agriculture has authority to seize animals which are in interstate or foreign com-
merce that are infected or have been exposed to contagious disease, and to destroy
these animals under certain conditions. This bill would extend the Secretary’s
authority upon the declaration of an extraordinary emergency so that he could
seize or destroy animals on any premise in the United States. This latter authority
exists within the states themselves and it is presumed that this would be used by
the Secretary only in case the states fail to act. The United States Livestock
Sanitary Association did not express opposition to this legislation at the time of
its introduction, but the Committee believes that some thought should be given to
this proposal, and the Association’s feeling in the matter should be clearly stated
for the Committee’s guidance.

Congressional Action: Not called for hearings. Congress adjourned without
taking action.

S-3176. Was introduced into the Congress providing for mandatory inspection
for wholesomeness on poultry and poultry products offered in interstate and foreign
commerce, and establishing a Poultry Inspection Section in the Federal Food &
Drug Administration. The United States Livestock Sanitary Association opposed
this bill.

Congressional Action: Bill killed in Committee, Substitute Bill submitted by
Committee (S-3933).

S-3588, S-3983, H. R.-11245, H. R.-11370, H. R.-11411, H. R.-10527 and H. R.-
10807. Bills introduced in either the House or Senate dealing with the mandatory
inspection of poultry and poultry products offered in interstate or foreign com-
merce. These bills differed in the type of inspection required, the agency designated
to do the job, and in other minor ways. The United States Livestock Sanitary
Association opposed all of these bills and on those scheduled for hearing filed with
the committee statements outlining our position and our reasons.

Congressional Action: None. Congress adjourned without taking action.

H. R.-11458, H. R.-11800. Were introduced and provided for amendment to the
Meat Inspection Act of 1906 to include—poultry and poultry products. As these
bills were consistent with the position taken by the United States Livestock Asso-
ciation, both of them were indorsed by this Association.

Congressional Action: None. Neither bill was called for a hearing before adjourn-
ment.

On April 18, 1956, the President of the United States sent to Congress a com-
munication requesting the addition of $18,915,000 to the budget for the Department
of Agriculture for 1957 to provide animal disease laboratory facilities at Beltsville,
Maryland. This matter was heard before the Senate Appropriations Committee,
and the United States Livestock Sanitary Association indorsed the item. The
Committee indicated general approval of the request, but declined to provide the
facilities at Beltsville, Maryland. As a result of this, and with the approval of the
Congress, a committee was appointed by the Secretary of Agriculture to recommend
an alternate site. The recommendation has been made and a site adjacent to the
Iowa State College has been selected.

It is a difficult and time consuming task to keep up with all of the legislation
introduced into the Congress affecting the Association and dealing with livestock
health matters. Appearing before Congressional committees on behalf of the Association is always at a time of the Committee's selection, and frequently results in great inconvenience. Your Committee is most anxious that the United States Sanitary Association express its views to the Congress on these matters, and if the 84th Congress is any indication of the future attitude the Congress is most anxious to have our opinions. It is the Committee's feeling that your Association has definite responsibility to its members, and to the livestock industry in this sphere of operation. The Committee recommends that your Association give serious thought to the establishment of representation in Washington for just this purpose.
REPORT OF COMMITTEE ON MORBIDITY AND MORTALITY

J. R. HAY, Columbus, Ohio, Chairman; W. L. BENJAMIN, Richmond, Virginia; C. G. BRADY, Ithaca, New York; L. O. EMIN, Atlanta, Georgia; H. E. GOLDSTEIN, Columbus, Ohio; R. A. HENDERSHOTT, Trenton, New Jersey; H. MARSH, Helena, Montana; A. P. SCHNEIDER, Boise, Idaho; F. A. TODD, Arlington, Virginia

Previous Committees on Morbidity and Mortality have assigned tasks for succeeding committees to follow. Among these assignments the following were included:

1. Expansion and development of organized state livestock disease reporting programs.
2. To urge the adoption of a National Livestock disease reporting program.
3. The development of a standardized list of reportable diseases.
4. To develop a manual of reportable diseases, as a companion to the Foreign Animal Disease Manual.

Your 1956 Committee on Morbidity and Mortality reports the following:

1. As of this date thirty-one states have organized animal disease reporting programs. Programs are being developed in three states, twelve states are considering reporting programs, while only two states have not considered such an undertaking.
2. In July of 1956, the Agricultural Research Service, United States Department of Agriculture, instituted a National Animal Disease Reporting Program.
3. In 1954 your Committee on Morbidity and Mortality recommended a list of reportable diseases which were adopted by the executive committee. This list was further expanded by recognizing additional disease conditions by action of the 1955 committee.
4. Your present committee has prepared an abstract of last year's report, Reportable Diseases of Domestic Animals, which at this time will be turned over to the Secretary for publication at such time as the Executive Committee deems advisable.

It has been most gratifying to be able to state that the assignments as set forth by previous committees has been completed. However, your present committee would like at this time to restate several points. First, the Agricultural Research Service should strive to expand their present National Reporting Program to incorporate those sources of information now available from the states with well organized disease reporting programs. Second, that the remaining states which do not have organized reporting programs be urged to do so at the earliest possible date. Third, that the Executive Committee consider printing the manual of Reportable Diseases of Domestic Animals at such time as they consider it economically sound; however, a temporary committee on publication should be appointed to review, correct, and incorporate that material which is applicable to and resulting from present and future research.

With these recommendations your present committee feels that its assigned task has been completed and it recommends that the Committee on Morbidity and Mortality be discontinued.
REPORT OF THE COMMITTEE ON PUBLIC RELATIONS


The activities of your Public Relations Committee as in previous years were directed in an effort to bring information about the United States Livestock Sanitary Association to the members of the livestock industry of this Country.

It has been learned that no matter how sound and basic the material to be presented is, it must have news appeal to attract attention.

Prior to this meeting a copy of the program together with a covering letter was forwarded to the members of the American Agricultural Editors Association. In the letter an invitation was extended to attend the meeting, at which time arrangements would be made for interviews with program speakers of their choice. They were also furnished the name and address of the State and Federal regulatory officials in their respective states and advised to contact them for any premeeting material or information concerning the United States Livestock Sanitary Association.

In connection with this a letter was forwarded to the regulatory officials in such states advising them of the action taken. To date several requests for program papers to be published have been received.

Public Relations activities during this meeting started last Sunday when an appearance was made before the annual meeting of the National Association of Television and Radio Farm Directors, advising them of the meeting and inviting them to attend.

On Monday a live radio broadcast was made at which time the functions and activities of this Association and its relationship to the Livestock Industry was explained.

During the remainder of the meeting the following topic material was presented either live or recorded by individuals or panel groups.

Brucellosis, Hog cholera, Sheep scabies, Foreign Animal Diseases, The Use of Antibiotics in Disease Control, Veterinary Education, and Meat Inspection.

In all of these presentations and discussions the theme was centered on the benefits the Livestock Industry receives from disease control and eradication programs and emphasis was placed upon the important part the livestock owner has in making control and eradication programs work.

It is urged that all regulatory officials make constant efforts to develop information outlets to explain the aims and objectives in animal disease control and eradication and the accomplishments and progress that has been made.

Your Committee recommends that consideration be given to placing a reference copy of each years proceedings in the hands of the principal Farm and Livestock publications and Radio and Television Farm Directors.
COMMITTEE ON EFFECT OF RADIOACTIVE MATERIAL ON LIVESTOCK


The potential danger to animals, as well as man, associated with radioactive materials seem to provoke concern to a much greater extent than other man-made hazards. The problem is not one of refuting these potential dangers, but one of intelligent and objective appraisal.

Nuclear fission products, particularly those associated with weapons testing fallout, constitute the radioactive materials causing the most public concern. It should be pointed out that in coming years similar problems may be associated with nuclear reactor wastes.

Atomic fallout can be considered a two phase problem: (1) possible acute or short term effects, and (2) the chronic or long term effects.

The committee calls attention to the fact that it is improbable that any evidence of acute radiation injury in livestock may be observed except in the proximity of the nuclear detonation.

The trial of C. D. Bullock, et al., vs. The United States of America held in Salt Lake City, Utah, during September of 1956 is pertinent to this report. It was the first contested legal action in which it was claimed that livestock had been injured from atomic fallout.

The plaintiffs claimed that unusual sheep losses from causes undetermined by livestock authorities and occurring coincidental to nearby nuclear weapons testing were directly or indirectly attributable to these tests.

The defense asserted that the radiation tolerances of sheep are reasonably known and that the maximum dose to which any sheep could have been exposed was many times lower than tolerance dose.

It was contended further that the pathology of irradiation damage is known and the tissues taken from the affected sheep were not characteristic of radiation injury.

The contentions of the defense were substantiated by voluminous testimony of experimental nature. Four members of the reporting committee participated in this trial.

The judge, Hon. A. Sherman Christenson, concluded that the preponderance of evidence indicated that the atomic fallout to which the animals might have been exposed was substantially less than would have caused acute damage to livestock. The lesions and signs of affected animals were not characteristic of radiation injury. The case was therefore dismissed.

At the present time (1) it is estimated that there is a total of 22 mc/mile² of Sr⁹⁰ in the soils of midwestern United States. Most of this is contained in the top two inches of soil.
This represents a level of about 0.04 MPC.* Alfalfa contained 0.018 presumably grown in similar soil as samples. Milk, on the other hand, was found to have about 0.008 MPC of Sr<sup>85</sup>. At the present time these levels are not considered hazardous to livestock. Criteria meant to apply only to people were used during evaluation. Since the economic life of domestic animals is shorter than the mean human life span, their radiation tolerances may be assumed to be higher. Sheep, for example, have lived a considerable part of their life span with a body burden of 100 times the MPC without noticeable evidence of injury or demonstrable blood changes. (2)

Radioiodines, like stronntium, are critically observed nuclides. They are fission products that are easily dispersed and are concentrated by thyroidal uptake of animals. Recently the newspapers carried a report of radioiodine contamination of Australian cattle 400 miles from the English weapons testing site.

Approximately 50 m <sup>131</sup> c/gm were found in thyroids of cattle from the vicinity of Memphis, Tennessee, in July 1955 (3). As a relative figure, it is pointed out that this is about 200,000 times less than used in human thyroid therapy.

At the Geneva Conference in 1955 it was reported that dairy cows could safely consume up to 2.3 <sup>131</sup> c of iodine daily (4). This would cause deleterious effects neither to the cow nor to those drinking her milk. Sheep have been fed 1.5 <sup>131</sup> c/day for two years without histological damage to thyroid (5). As much as five milli-curies have been given to sheep at one time with only mild radiation damage to the thyroid gland (5).

Realistic field values or standards which are applicable to livestock or animal products have not yet been developed. A general awareness of the necessity of objective standards should initiate research in this direction in the near future.

REFERENCES

(2) COMAR, C. L.: Special Section, Annual Report UT-AEC, ARP, 1954.
(4) CHAMBERLAIN, A. C., LOUTIT, J. F., MARTIN, R. P., AND RUSSELL R. SCOTT: The Behavior of I-131, Sr<sup>85</sup> and Sr<sup>89</sup> in Certain Agricultural Food Chains. A/Conf. 8/P/393.

* MPC-1 microcurie of radiostrontium per kilogram of calcium is maximum permissible concentration.
The committee on Regulatory Education wishes to state that no adverse comments were received relative to the recommendations outlined in last year's report, and in general we are inclined to believe that the report was well received. Coming as it did, late in the school year, there was little reason to expect that any curricular changes could be made in our veterinary schools to give more time to this part of the student training program.

Nevertheless, a letter was sent to each of the Deans of the Veterinary Schools and to each of the chief state regulatory officials in states having veterinary schools. The purpose of this letter was two fold in that it would serve as a subtle reminder and incidentally might stimulate rereading of the recommendations made by this committee in its report of last year, and at the same time provide information which would indicate what if any progress was being made.

 Replies were received from 14 of the veterinary deans. Five reported that increased emphasis was given to regulatory education during this past year and two deans reported plans for changes in curriculum to provide on-the-job training in the near future. In six of the schools freshmen students are given an introduction to regulatory veterinary medicine as a part of the survey course covering all phases of veterinary medicine. Five other schools begin with the 3rd year or junior class, in presenting the subject of regulatory medicine.

 Only four schools reported that representatives of the Washington Office of A. R. S. had appeared before the student body during the past year.

 All except two schools reported that state and federal veterinarians appeared before their senior students to discuss regulatory veterinary medicine.

 Of the 14 chief regulatory officials reporting, 13 stated that they had been requested and had assisted with the regulatory education program in the veterinary colleges in their respective states.

 Seven of 14 reported the use of demonstration materials or on-the-job training as part of the program. The amount of time devoted to the program by these state or federal men, varied from one hour to three weeks; the majority were from one half to two days. Ten of the 13 reported that educational kits, covering regulations, required forms and instructions for their preparation, state and federal regulations for interstate movement of animals etc. were given to senior students and to all applicants for licenses.

 The answer to specific questions and supplemental comments received in the replies from the deans and the state veterinarians, indicate that there is an increased awareness of the need for greater emphasis in the broad field of regulatory education in the training of veterinary students; in addition, the fine spirit of the state and federal regulatory officials as shown by their willingness to contribute their time and the use of their facilities is commendable and represents real progress.
Your committee wishes to again remind the membership that veterinary medicine encompasses a broad field of activities in which veterinary students must be indoctrinated during their course of training to become veterinarians. It is impossible for the schools to develop students to the point of maximum proficiency in any area of professional activities during the time allotted for veterinary education. The thing which we should strive for is a sound basic understanding and above all the proper attitude, and a full appreciation of the importance of all phases of veterinary activities. With this type of approach, all areas will receive proper recognition and in turn society will be best served by our profession.

Your committee wishes to again endorse the recommendations submitted in the report of last year. We would particularly like to urge the officials of the ARS in Washington to make more frequent visits, to the veterinary colleges. In the opinion of the committee such visits are highly essential in stimulating an awareness and proper regard for regulatory work.

We also want to urge the one or two schools, who do not now utilize the services of their state and federal regulatory officials in their student training programs, to give some thought to the need and means of supplementing their present program of instruction in the field of regulatory education.
REPORT OF COMMITTEE ON RESOLUTIONS

Dr. J. Walter Hastings, Sr., Cambridge, Maryland, Chairman; Dr. R. W. Carter, Columbia, South Carolina; Dr. J. S. Campbell, Little Rock, Arkansas; Dr. T. J. Grennan, Jr., Providence, Rhode Island; Dr. L. L. Breeck, Frankfort, Kentucky; Dr. F. B. Wheeler, Baton Rouge, Louisiana

Resolved by the United States Livestock Sanitary Association in the 60th Annual Meeting assembled in Chicago, Illinois, November 28, 1956:

That the United States Livestock Sanitary Association convey its sincere thanks and appreciation to each speaker on the program and to each member of the several committees for their services in the preparation and presentation of this 1956 program.

That we express our sincere thanks and commend our Secretary, Dr. R. A. Hendershott for his excellent and untiring efforts in behalf of this organization.

That the United States Livestock Sanitary Association convey its sincere thanks and appreciation to Charlotte Emmons for her excellent service in annually recording the meetings of the Association.

RESOLUTIONS REFERRING TO MASTITIS CONTROL

WHEREAS, mastitis has long been recognized as a major disease problem affecting a great number of our dairy cattle, and

WHEREAS, uncontrolled sales of antibiotics are available for uncontrolled and often ill-advised treatment of this disease, and

WHEREAS, it has been shown that the presence of antibiotics create a definite economic problem when present in fluid milk used for cheese manufacture, and it is found that the repeated exposure of traces of antibiotics may present a definite detriment to public health:

Therefore, Be It Resolved by the United States Livestock Sanitary Association duly assembled at the Morrison Hotel, Chicago, Illinois on November 28-29 and 30th, 1956, request that the United States Pure Food and Drug Agency demand that all products containing any antibiotic (so-called) used for the treatment of mastitis, incorporate a harmless dye substance which by its presence will eliminate the possibility of milk containing the above product reaching fluid milk channels to be used for manufacture or human consumption.

RESOLUTION REFERRING TO MASTITIS RESEARCH

WHEREAS, mastitis in dairy cattle is estimated to cost milk producers upwards of $200 million a year; and

WHEREAS, there is still no uniformly successful method of preventing or controlling this disease; and

WHEREAS, the presence of antibiotics in milk as a result of treating cattle for mastitis is of increasing concern to producers, distributors, and health officials;

Therefore, Be It Resolved, that the United States Livestock Sanitary Association in convention assembled at Chicago, Illinois November 28-30, 1956, urges
the United States Department of Agriculture to augment its funds for research on mastitis independently and in cooperation with the states in order that this costly disease may be more successfully prevented and controlled.

RESOLUTION REFERRING TO LEPTOSPIROSIS CONTROL

WHEREAS, leptospirosis has been demonstrated in several species of livestock in all areas of the United States, and

WHEREAS, there is no uniform approved diagnosis, treatment, and control procedures on the local State and National level:

THEREFORE, Be It Resolved by the United States Livestock Sanitary Association, assembled in Chicago, Illinois, November 28, 29 and 30th, 1956, that the United States Department of Agriculture plan, coordinate and execute research directed to the diagnosis, treatment and control of leptospirosis, so that uniform procedures can be formulated in all states.

RESOLUTION REFERRING TO HOG CHOLERA SUPPRESSION

WHEREAS, for over 100 years this nation has been forced to live with hog cholera which has been extremely costly and is contrary to the American principle of eradicating animal disease, and,

WHEREAS, the development of methods for immunizing swine against hog cholera have been developed which do not require the use of fully virulent virus which is a means of perpetuating the disease; and

WHEREAS, the development of these new hog cholera vaccines seem to give promise for use in more effectively controlling and eventually eradicating hog cholera:

THEREFORE, Be It Resolved that the United States Livestock Sanitary Association, in convention assembled at Chicago, Illinois, November 28–30, 1956, urges the federal government to work with the states in developing a plan for controlling and eradicating hog cholera; and

Be It Resolved Further, that if it develops that there is need for additional information in regard to this disease before proceeding with the eradication program, that research and trial projects be conducted to produce the information needed.

RESOLUTION REFERRING TO MANDATORY COOKING OF GARBAGE FOR SWINE FEEDING

WHEREAS, the cooking of garbage being fed to swine has proven to be an effective and important factor in the control and eradication of vesicular exanthema; and

WHEREAS, it is known that the cooking of garbage fed to swine is also an important factor in the control of hog cholera and other swine diseases; and

WHEREAS, the laws and regulations requiring the cooking of garbage fed to swine are being successfully enforced:

THEREFORE, Be It Resolved, by the United States Livestock Sanitary Association in convention assembled at Chicago, Illinois, November 28–30, 1956, that
the states and federal government be requested to continue their programs requiring the cooking of garbage fed to swine.

RESOLUTION REFERRING TO SWINE BRUCELLOSIS

WHEREAS, brucellosis in swine is a serious and costly disease confronted by swine producers; and
WHEREAS, the fine progress being made in the control of bovine brucellosis tends to focus attention on brucellosis in swine as an animal disease and public health problem; and
WHEREAS, the infection of human beings from contact with swine infected with brucellosis constitutes a serious menace to public health:

THEREFORE BE IT RESOLVED that the United States Livestock Sanitary Association, in convention assembled at Chicago, Illinois, November 28-30, 1956, urges the United States Department of Agriculture to develop and recommend to the states a program to be cooperatively supported and financed for the control and eradication of swine brucellosis.

RESOLUTION REFERRING TO SCRAPIE ERADICATION

WHEREAS, Scrapie, an infectious nerve disease of sheep first identified in this country in April, 1947, continues to spread; and
WHEREAS, this infection, believed to be caused by a filtrable virus, now has been found in 50 flocks located in 17 states; and
WHEREAS, the continued spread of Scrapie is a dangerous threat to the economic welfare of the nation's sheep industry;
WHEREAS, Scrapie is a continuing menace to the welfare of our sheep industry, we reaffirm our determination not to live with this insidious and economically important disease:

THEREFORE, BE IT RESOLVED, by the United States Livestock Sanitary Association, in convention assembled at Chicago, Illinois, November 28-30, 1956, that the Secretary of Agriculture be requested to undertake additional research on the nature of this disease and to strengthen present regulatory requirements in order to control more effectively and eradicate Scrapie among the sheep of this country.

RESOLUTION REFERRING TO TRAINING COURSES IN REGULATORY AND INSPECTION WORK

WHEREAS, many professional employees of the United States Department of Agriculture have special training and competence in various inspection and regulatory programs; and
WHEREAS, many state colleges and universities desire to have such employees participate in special courses of instruction for students in these professional fields:

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Association in convention assembled at Chicago, Illinois, November 28-30, 1956, requests the Secretary of Agriculture to formulate and propose, and requests the Congress to pass legislation to authorize the Department to respond to requests of colleges and universities to assign professional employees to part-time teaching
assignments with reference to subjects within their areas of competence, and pro-
viding that the college or university will defray the entire cost of such assignment.

RESOLUTION REFERRING TO STATE MEAT INSPECTION

WHEREAS, only approximately 80 percent of animals commercially slaughtered
come under federal meat inspection; and

WHEREAS, there is need for additional inspection covering the other 20 percent
of animals slaughtered commercially to protect consumers and producers, and

WHEREAS, it has been demonstrated that successful meat inspection programs
can be administered by state departments of agriculture:

THEREFORE, BE IT RESOLVED, that this United States Livestock Sanitary Asso-
ciation, in convention assembled in Chicago, Illinois, November 28–30, 1956, does
urge the states to enact legislation which provides for an adequate program of in-
spection of animals to be slaughtered for food not covered by federal inspection,
and to include anti- and post-mortem inspection of the individual animals under
veterinary supervision.

RESOLUTION REFERRING TO COMPULSORY INSPECTION OF POULTRY

WHEREAS, there have been several bills introduced in Congress pertaining to
the compulsory inspection of poultry; and

WHEREAS, some of these bills delegate the authority for administration of com-
pulsory inspection of poultry to various services or departments of the federal
government:

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Asso-
ciation, in convention assembled at Chicago, Illinois, November 28–30, 1956, urges
Congress to place the administration of compulsory inspection of poultry in the
Meat Inspection Branch, United States Department of Agriculture.

RESOLUTION REFERRING TO A FEDERAL REGULATION PERTAINING TO
THE INTERSTATE SHIPMENT OF SHEEP FROM SHEEP SCAB INFESTED STATES

WHEREAS, sheep scab is a costly parasitic disease which costs the livestock in-
dustry vast sums of money in the control thereof and even more costly in loss of
wool, gain, and even life; and

WHEREAS, certain of the states are free of the infestation and are spending much
time, effort and money to remain free; and

WHEREAS, a simple and relatively inexpensive means of eradicating the disease
is available; and

WHEREAS, certain areas in the United States remain as foci of infection and
sheep from these areas continually find their way to clean areas in spite of impor-
tation regulations of the various states.

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Asso-
ciation in the 60th Annual meeting, assembled in Chicago, Illinois, November 30,
1956, request the Secretary of Agriculture, through the Animal Disease Eradica-
tion Branch of the Agricultural Research Service to promulgate a regulation to
prevent the movement of sheep from states where sheep scab has been known to
exist within the past twelve months, unless they are properly dipped, under of-
ficial supervision, immediately prior to shipment, or unless consigned for immediate slaughter.

This regulation should apply to all terminal markets which receive sheep from infected states, except that sheep from non-infected states may be handled without dipping provided that clean and disinfected pens are available, and such sheep are kept separate and apart from all others, and when transported, are loaded in clean and disinfected conveyances.

Sheep from states proven to be free from sheep scab for the past twelve months or more, may move freely, except when passing through terminal markets which do not comply with paragraph two (2) above.

Sheep may be unloaded under the twenty eight (28) hour law in pens and corrals, only if such pens have been cleaned and disinfected as provided by Federal law.
PROPOSED AMENDMENT TO THE CONSTITUTION AND BY-LAWS

R. W. SMITH

Concord, New Hampshire

Dr. R. W. Smith: Your Committee has attended to the duties assigned to it, and offers this amendment to the Constitution of the United States Livestock Sanitary Association.

Amend Article III, Membership, by striking out the first line in said Article and substituting the following: "There shall be three kinds of members—official, individual, and nonvoting junior."

Also, add after the ninth line in Article III the words, "... and nonvoting junior", so that said Article as amended will read as follows:

"Article III—Membership: There shall be three kinds of members—official, individual, and nonvoting junior. The livestock sanitary departments of each state, also the United States and the Canadian, Cuban and Mexican governments, the Territories of Puerto Rico, the Virgin Islands, and Los Angeles County, California, shall be eligible to official membership in this Association, and shall be represented on the Executive Committee by the livestock sanitary executive official. Any person engaged in livestock sanitary work for federal, provincial, state, territory, county or municipal governments, and any other person interested in livestock sanitation of milk and meat hygiene, may be elected to individual and nonvoting junior membership."

This was concurred in by the other two members of the Committee, Dr. West and Dr. Schneider of Idaho. As set forth in another section of the Constitution, this matter must lie on the table until next year, and will come to a final vote at that time.
REPORT OF THE COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION

A. Z. Baker, Chairman, Cleveland, Ohio; Dr. I. W. Cole, A.D.E., A.R.S., Washington, D. C.; R. L. Cuff, Kansas City, Mo.; Dr. G. H. Good, Cheyenne, Wyoming; A. G. Pickett, Topeka, Kansas; Earle G. Reed, Omaha, Nebraska

This Committee is concerned with the facilities and services furnished by stockyard owners, market operators and carriers in the transportation and marketing of livestock; and with the regulations and instructions issued and administered by regulatory agencies of the Federal and the various State Governments to control and prevent the spread of livestock diseases.

This Committee notes with satisfaction and commends the Federal and State regulatory officials for the development of realistic regulations and instructions for the effective control of livestock diseases. Consideration has been given to the problems and the impact of such regulations and instructions on producers, carriers, stockyard owners, market operators and feeders, and for the cooperative and effective manner in which such regulations and instructions have been administered and enforced.

This Committee notes with gratification the trend toward uniformity of disease control regulations and practices and suggests to the Association that it continue to work for greater uniformity. The Committee has been pleased with the willingness of livestock disease control officials to promptly modify regulations, instructions, and practices when experience and changed conditions indicated such modification would not unreasonably jeopardize the control or eradication programs. This procedure would relieve producers, carriers, stockyard owners, market operators, slaughterers and feeders from unnecessary and burdensome controls. It is believed that such willingness to modify regulations and practices will result in more active co-operation of all segments of the livestock industry in the necessary programs.

The Committee is pleased to join others of the association in satisfaction with the substantial freedom from widespread livestock disease epidemics, but wishes to voice a warning against complacency on the part of disease control officials and others of the livestock industry. This Committee is especially conscious of the danger of the rapid spread of livestock diseases in view of the multiplicity of transportation and marketing agencies, many of which operate under limited inspection, supervision or control.

This Committee has been gratified with the absence of retaliatory regulations among the several regulatory agencies and between states, and feels that the Association has had and can continue to play an important part in encouraging cooperation among all concerned with livestock disease control and eradication.

The Committee is gratified with the success of the programs for the control and eradication of Vesicular Exanthema, which has been greatly aided by the cooperation of the Federal and most of the State authorities. The producers, carriers, stockyard owners, market operations and slaughterers have cooperated
splendidly. The recent outbreaks of the V.E. disease in one of the Eastern states indicate the need for further and more vigorous enforcement of recognized practices, particularly the cooking of all garbage fed to swine, in order to completely eradicate the disease.

The livestock industry, for many years, has been living with two livestock diseases—Hog Cholera and Sheep Scabies which many authorities consider could now be practically eradicated by a comprehensive and vigorous attack. The whole industry, including that part engaged in transporting and marketing swine and sheep, have been carrying a tremendous cost load in the control programs with which they have been burdened. The Committee believes, and, therefore, suggests to the Association that it support an intensive study of the possibilities for the eradication of these diseases.

And the Committee further feels that some of the practices, particularly the annual cleaning and disinfecting of stockyards, enforced at some stockyards and not at others, is of questionable value or necessity in view of the experience in the control of sheep scabies, and the basic requirements. That reasonable stockyard services require the furnishing of suitably cleaned pens at all times and a complete cleaning and disinfecting following any use by diseased or exposed livestock. This Committee recommends that the proper committee of the Association consider some more appropriate program for the eradication of sheep scabies.

The Committee recommends that the Association request all regulatory agencies to review and, where justified by experience or conditions, modify, revise or eliminate regulations, instructions and practices pertaining to the cleaning and disinfecting of transportation vehicles, stockyards and market facilities used in the handling and movement of livestock.

In view of the new regulations relating to Brucellosis which are to be made effective January 1, 1957, the Committee recommends that the Association urge the Federal authorities to make every possible and reasonable effort to acquaint all segments of the livestock industry generally that are directly affected with the provisions and the requirements of the regulations; to be as tolerant as possible in the administration of the regulations at best until the industry has had a reasonable opportunity to become familiar with their requirements; to be prepared to modify the regulations or the administrative practices when and if experience or changed conditions justify such modification.

The Committee feels that there is an urgent need for a simple compilation of the requirements of all destination states to enable carriers, stockyard owners and market operators to comply with regulations without uncertainty, expensive inquiry, or unnecessary delays.

The Committee submits this report and recommends that it be received and referred to appropriate committees for implementation.
A PRACTICAL EVALUATION OF LIVE IMMUNIZING AGENTS

J. M. HEJL, D.V.M.

Washington, D.C.

Live viruses, modified live virus vaccines and attenuated living biologics in general are good immunizing agents but they have created some problems for State regulatory officials in disease control work. How do these new products fit into plans of disease control? I hope that what I have to say will be of some help in this matter.

What are live virus and modified live virus vaccines? A live virus vaccine is one prepared from a live viral agent usually isolated from an infected animal or bird. The agent is virulent and capable of reproducing disease. It may reproduce disease either with ease or difficulty and in some instances when administered by an unnatural route of exposure it may not even cause disease. Major biologics of this type are: Fowl laryngotracheitis vaccine, Newcastle disease vaccine, Infectious Bronchitis vaccine, Fowl-Pox vaccine, Pigeon-Pox vaccine and Hog-cholera virus. A live virus vaccine may be avirulent which means that the strain of virus used in the preparation of the product, although showing some characteristics of the typical virus, is atypical in disease-producing potential. Usually the virus is naturally attenuated and avirulent or, at the most, produces a mild disease condition. Such a vaccine is the Intranasal type of Newcastle disease vaccine, commonly referred to as the “B, Strain”. Similar products of bacterial origin are: Brucella abortus vaccine, Strain 19 and Erysipelas vaccine, live culture, avirulent.

A modified live virus vaccine is one prepared from a virus whose disease-producing property has been altered but is still capable of stimulating an antigenic response. Viruses are modified by repeated passage in unnatural hosts, such as the passage of Hog-cholera virus in rabbits, in chick embryos and in tissue culture. Major biologics of this type are: Hog-cholera vaccine, modified live virus, rabbit and porcine origins; Rabies vaccine, modified live virus, chick embryo origin; Bluetongue vaccine, modified live virus, chick embryo origin; and Canine distemper vaccine, modified live virus, chick embryo origin.

The shift in production and use of veterinary biologics from inactivated vaccines, bacterins, and serums to live and modified live virus vaccines created some new problems and made us reexamine the methods used to evaluate a product for licensing. I am sure this same situation faces the State official when considering such products for use in disease control programs. For a practical evaluation of these products one must know what to expect from the preparations and three main points must be considered—potency, safety and the diseases against which the products will be used.

Live and modified live virus vaccines will stimulate an immunity which is more rapid in onset and of longer duration than that from some of the counterpart inactivated preparations. Repeated vaccinations usually are not necessary. These are assets to a biologic.

It is of common knowledge that the modified live virus vaccines cause reactions
in some vaccinated animals evidenced by leucopenia and mild febrile response especially in highly susceptible animals or animals in a lowered health status and maintained under poor husbandry conditions. Because of this type of reaction the modified live virus vaccines are not absolutely safe, but these reactions are usually significantly low when compared to the total number of animals vaccinated. Animals in lowered health status and in surroundings of poor husbandry are not good subjects for vaccination. Such unknown factors as highly susceptible animals or highly susceptible herds will be occasionally encountered and in these exceptional cases trouble can be expected. No biologic is 100 percent perfect and trouble cases are inevitable. Personal preference of the practitioner I am sure is divided between inactivated products and modified live virus products. Inactivated products are safe requiring two to three weeks to stimulate an immune response which may last for a period up to a year and repeated vaccinations may be necessary while the modified live virus vaccines, although possessing a high degree of safety, are not absolutely safe but stimulate an immunity which is more rapid in onset and of longer duration.

Live and modified live virus vaccines have been incriminated, sometimes unjustly, for exciting or “triggering off” latent infections harbored by animals at the time of vaccination. To the best of our knowledge this has not been proven to be true following the use of modified live virus vaccines. On the other hand it is possible following the use of live virus poultry vaccines and it has been shown rather conclusively that live virus Newcastle disease vaccine and Infectious Bronchitis vaccine can be “triggering off” mechanisms of latent PPLO infections in poultry. Sometimes it is impossible to determine whether birds or animals harbor latent infections, and in other cases it is overlooked. Checking the history and health status of the flock or herd before vaccination still remains important to successful vaccination. Too, there is evidence that modified live virus vaccines interfere with normal fetal development. This could hardly be classed as a risk because if precautions are taken not to vaccinate during gestation, this feature can be circumvented.

When evaluating a modified live virus vaccine there is always the question, will the vaccine revert to full virulence? From an analysis of data submitted in support of license applications for the modified live virus vaccines mentioned earlier, reversion to virulence did not occur. Shedding of the modified virus by vaccinated animals could not always be demonstrated and in other instances where there is a shedding, transmission to susceptible contact animals did not always take place. However, with the modified live virus Hog-cholera vaccines there is some shedding and transmission of the modified virus to susceptible contact animals. Contact animals in which there is generalized invasion of the modified virus are usually resistant to exposure with virulent Hog-cholera virus. But what happens when these contact animals shed and transmit the virus to other susceptible contacts and such a series continues? In an experiment designed to determine whether a high passaged rabbit origin vaccine would revert to virulence, it was found that the virus did not revert to virulence in eleven back passages. However, it is conceivable that some of the modified live virus Hog-cholera vaccines could be made
to revert to virulence by laboratory experiments under ideal conditions and specifically designed for that purpose.

Earlier I mentioned that Potency, Safety and the disease against which a product will be used must be considered in a practical evaluation of the product. I will use the disease hog-cholera and the different types of products recommended for its control as an example to illustrate these three factors. The evaluation of other live virus and modified live virus vaccines could be analogous in many respects to this example.

Hog cholera is enzootic in certain areas of the country and either of low incidence or absent in other areas. In areas where hog cholera is prevalent the simultaneous use of virulent virus and antisera proves to be a satisfactory means of immunizing animals. A drawback to the use of this method of vaccination is that the disease is continually perpetuated. The disease can be controlled by the use of live virus but no hope exists for eradication or even reducing the incidence of disease. The modified live virus hog cholera vaccines are being used more and more in enzootic areas and even in other areas where the disease is not of such prevalence and with their use there is hope of reducing the incidence of disease. The vaccines stimulate an immunity rapid in onset usually within seven days and when administered with an adequate dose of antiserum, protection occurs sooner. This immunity is of long duration, generally lasting two to three years. In enzootic areas occasional trouble cases may be of secondary importance and overshadowed by the need of a biologic which stimulates a rapid, durable and long lasting immunity. In areas where the incidence of the disease is either low or absent occasional trouble cases may be significant especially since these cases probably could have been avoided with the use of an inactivated vaccine. In these latter areas there isn’t the risk of exposure to hog cholera during the several weeks which it takes for an inactivated vaccine to stimulate an immune response. Hence selecting the biologic of choice depends on the conditions at hand and on personal preference.

There are other sections of the country where hog cholera is almost totally absent. In at least eight States, virulent hog cholera virus has been outlawed to prevent the introduction of the disease through its use. Analogous to this situation of introducing disease is the use of live virus poultry vaccines.

There are indications that live virus poultry vaccines can cause disease in susceptible birds. This factor sometimes disregarded in disease control, may be of secondary importance in densely populated poultry areas because the more common poultry diseases are already rampant in these areas. It becomes of primary importance to maintain an immune status in birds with as rapid an onset as possible. Of equal importance, at least to the flock owner, is the cost of vaccines and the time saved by methods of mass administration to which the live virus preparations are best suited. This situation, I’m sure, doesn’t exist in all parts of the country. In many states, poultry raising, although on the increase, still is a means of supplemental farm income and a source of the family’s private needs for meat and eggs. Too, some areas, because of geographical features, extent of poultry population, and others, have not experienced typical poultry diseases and the
mixed infections to the degree that others have; in fact, some diseases are absent. In those areas, caution should be taken when considering the use of live virus poultry vaccines for routine preventive vaccination. The selection and recommendation of a vaccine in poultry disease control programs should depend upon the conditions at hand. State regulatory officials in those areas not experiencing problems with Newcastle disease and Infectious Bronchitis should recognize that these diseases are airborne and easily transmitted and that with the use of these two live virus vaccines, it is possible to introduce and disseminate the viruses.

Safety and potency of a live or modified live virus product are important factors considered before such products are licensed. Standards and safeguards are applied in the evaluation of the biologic and if unknown, they are developed from an analysis of the data presented by the license applicant and from that published by research workers. Licensees are not authorized to distribute a product even for experimental purposes unless preliminary laboratory investigations indicate that the product is safe and has promise as an immunizing agent. If this can be demonstrated, the product can be released for field evaluation under certain conditions. First, the State official involved must give his permission to the manufacturer to carry out the studies in his State; the product must be labeled “for experimental use only—not for sale”; the firm’s plan of carrying out field studies must be furnished to the Branch, along with the approximate number of animals to be used, the premises involved, and the persons responsible for supervising the experiments. Adequate records must be maintained and results eventually must be furnished the Branch.

It remains the responsibility of the licensee to furnish data adequate for our evaluation for a determination in licensing. Depending on the nature of the product and especially if it is a new or relatively new biologic, the licensee is requested to conduct certain tests under the observation of Branch inspectors and on occasion, arrangements are made for testing by the Department.

In analysing laboratory and field data to determine the safety of living preparations, some of the factors receiving attention are:

1. The possibility of a vaccine causing disease, either typical or atypical,
2. The possibility of a vaccine causing reactions in vaccinated animals and the degree of severity of these reactions, and
3. The possibility of the vaccinated animals shedding the vaccine virus, carriers, and the reversion of a modified or attenuated virus to virulence.

When your committee on biologicals and pharmaceuticals asked that I prepare a paper on the practical evaluation of live immunizing agents for the benefit of the State livestock regulatory officials, it occurred to me that many of the State officials may not fully understand the Federal program of licensing biological products. The State official is responsible for the control of diseases in his respective State and to a large extent must cooperate with neighboring States in these matters. For that reason, he should be aware of the types of license issued and the reasons why such licenses are issued.

There are two types of United States Veterinary License which authorize the interstate distribution of veterinary biologics; namely, a Regular License and a Special License. Before either type of license is issued under the Virus-Serum-
Toxin Law, the applicant must demonstrate that the product under consideration is not worthless, contaminated, dangerous, or harmful. The selection and use of the words “regular” and “special” is administrative terminology, attempting to relay to the State officials and users of the product, especially in the case of a Special License, the conditions under which the product is licensed. Contrary to the beliefs of some, the word “limited” is no longer used for designating a type of license. A limited license is synonymous with “Special License”.

A United States Veterinary License without any specific conditions is referred to as a “regular license”. This means that the Department from data submitted in support of a license application, is satisfied that the product is safe for use and has worth and that it is eligible for interstate distribution.

A United States Veterinary License, “Special”, differs from a regular license in that certain conditions are imposed on the distribution of the product. Conditions of a special license are set forth under Section 102.7 of the Rules and Regulations Relating to Viruses, Serums, Toxins, and Analogous Products and to Certain Organisms and Vectors. Any one or all of these conditions may be applicable to licensing of a particular product. The Chief may impose other requirements to protect the livestock industry and other segments of the public when, in his opinion adequate protection will not be afforded by those specific conditions set forth in the Regulations. This authority is invested in the Chief because it is impossible to comprehend in advance the many possible variables and peculiarities of biological products.

The word “Special” should not be interpreted as a stigma placed on a product. Then why is such a license issued? There are many reasons, and I believe it can best be explained by citing examples. Methods of vaccinating swine to prevent hog cholera known as the simultaneous treatment with hog-cholera virus and anti-hog-cholera serum, crystal violet and tissue vaccines were in use for many years and the products were well established and proven. Then for the first time the modified live virus vaccines were proposed for licensing with the new biological concept of reducing the virulence of the virus while still retaining antigenicity through the adaptation of the virus in an unnatural host, such as the rabbit. All evidence indicated that this concept was true. The data submitted in support of the license application demonstrated the safety and efficacy of the vaccine, but these data were accumulated under laboratory conditions; and the field studies were relatively limited when compared to the proven and established products. A special license was issued with the main condition of the license requiring licensees to include a questionnaire with each package of vaccine. The questionnaire sought information from the practicing veterinarian and farmer on the results experienced following vaccination which were used in further evaluating the product under field conditions. The results of many thousands of vaccinations substantiated the earlier laboratory and field findings and shortly thereafter a regular license was issued.

Another condition of a special license, which probably is not fully understood by everyone, is that which requires manufacturers to distribute a product only in accord with the regulations or restrictions of the State of destination. This condition is used to advantage mostly with live virus vaccines under Special license,
such as Infectious Bronchitis vaccine. It protects those States which prohibit the entry of live virus vaccines, or where State regulations are in effect to control diseases, such as erysipelas, with a particular type of product. A special license with this condition brings to the attention of the State official that there may be some aspect of the product which he should investigate before authorizing entry and use of the product.

In summary, the live virus vaccines can be used in those areas where the presence of highly contagious diseases would cause severe losses if vaccination is not carried out. In some instances, especially for poultry vaccination, the live virus vaccines may be the only type of product available for this purpose. The modified live virus vaccines can be used with a high degree of safety. They appear to have a definite place in disease control programs. The inactivated vaccines, which have been used successfully in certain areas and under certain conditions, are probably best suited for vaccination in the later stages of eradicating a disease.
The Committee on Biologicals and Pharmaceuticals has attempted to review briefly the recent developments in these fields which in our opinion are of significance in the regulatory phases of disease control. One of the functions of each committee has been to arrange for one or more papers on the program. This committee limited its program material to one paper designed to be of immediate practical interest and value to men engaged in regulatory work.

Your committee is of the opinion that there is some confusion and doubt in the minds of some of those responsible for disease control as to the potency, safety, and effectiveness of several of the immunizing agents prepared from live or modified viruses and bacteria. Although at recent meetings of the United States Livestock Sanitary Association, these products have been discussed individually, it was decided that it would be profitable to present a brief review of the facts in regard to this group of products, with a practical evaluation of these immunizing agents from the standpoint of livestock sanitary officials, as an aid to them in deciding whether to approve certain products for control work under various conditions. Dr. J. M. Hejl, Chief of the Biological Products Licensing Section of the Animal Inspection and Quarantine Branch, consented to cover this subject in his paper entitled “A Practical Evaluation of Live Immunizing Agents”.

**BIOLOGICAL PRODUCTS**

During the past year there have been no outstanding new developments in immunizing agents that have reached the stage of being made available for general use, but some progress can be reported in improvements in some products, and added information has been obtained in regard to the evaluation of some of the newer products. The records of the Biological Products Licensing Section of the Agricultural Research Service show only three new licensed products since June 1955. This may be largely due to the wider use of chemo-therapeutic agents in a variety of ways for the prevention or treatment of animal infections. The use of immune serums of all types seems to be decreasing and several have virtually disappeared from the market. Agents producing active immunity, however, seem to be on the increase, but it is the opinion of your Committee that several of the products currently sold should be critically re-evaluated. Among the serums recently discontinued, it is interesting to note that Botulinus Antitoxin, Types A & B, Botulinus Antitoxin, Type A, Botulinus Antitoxin, Type B, and Botulinus Antitoxin, Type C, have been discontinued, apparently because of limited demand. However, one firm continues to produce a combination product, Botulinus Antitoxin, Type A, B, C. Recently the only firm producing Anti-Clostridium Hemolyticum serum has discontinued its production, again because of limited
demand. The loss of availability of this product is keenly felt in areas where bacillary hemoglobinuria is active.

There are several biological products in the immunizing group which we think deserve brief mention.

**Hog Cholera Vaccines**

The field of immunization against hog cholera continues to show changes. There now are eight states which have prohibited the sale and use of virulent virus. These states are Alabama, Georgia, Louisiana, Mississippi, Montana, North Dakota, Tennessee, and Wyoming. According to official information the field usage of virulent virus has decreased from approximately 40,000,000 doses in 1951 to 10,000,000 doses for the year ending June 30, 1956. This represents 28 percent of the total number of swine vaccinated.

During the past year 42 percent of all swine vaccinated against hog cholera received modified virus with a minimum dose of serum, while 25 percent of all swine vaccinated received modified virus without serum. Inactivated hog cholera vaccines represent only five percent of total product usage.

Data supplied by several state diagnostic laboratories indicate that both early and late breaks may occur following usage of all hog cholera prophylactics. In the great majority of instances these breaks appear related to factors present in the swine rather than in potency or protective value of the products used.

**Erysipelas**

Avirulent antigenic strains of *E. rhusiopathiae* reported by the Committee last year have now had extensive field usage. Surveys indicate that the results from avirulent erysipelas vaccine have been satisfactory. Antigenicity of the avirulent erysipelas vaccine is demonstrable by the skin scratch test on swine. The duration of protection has not been clearly defined but field results indicate that a single dose will carry pigs to market age. Results in field usage on turkey flocks have also been good.

In the midwest swine states there has been an increasing usage of either avirulent erysipelas vaccine or erysipelas bacterin on brood sows during early pregnancy. This practice appears to confer a substantial measure of passive antibody protection to suckling pigs.

**Anthrax**

In the 1955 report of this committee, the licensed production of Sterne anthrax vaccine was reported. Within the past year it was extensively used in a Mississippi anthrax outbreak and the protection of exposed cattle herds was satisfactory.

This product, prepared from an original culture developed at Onderstepoort, South Africa, possesses extremely low virulence with high antigenicity. An added feature is that the Sterne-type vaccine can be standardized and potency-tested on guinea pigs. It can be used on horses, sheep, goats, and swine with expectancy of a protective titer against field exposure within seven days.
Leptospirosis

Adequate diagnosis of suspected leptospirosis in the various species of livestock has been difficult because of the complex nature of laboratory blood tests.

A standardized *Leptospira pomona* antigen suitable for rapid plate or capillary tube testing is now licensed and commercially available. Similar rapid plate antigens for *L. canicola* and *icterohemorrhagia* should soon be available. Credit for development of this practical diagnostic antigen is due to Dr. H. Stoenner of the Rocky Mountain Laboratory, Hamilton, Montana.

Several brands of *Leptospira pomona* bacterins, grown on special media or in chicken embryos are now available to veterinarians. A combined *L. canicola* and *L. icterohemorrhagia* bacterin for protection of dogs has also been announced.

Currently, there is need for more extensive critical data on the adequacy and duration of protection conferred by *L. pomona* bacterins. It can be stated, however, that widespread clinical observations indicate appreciable value in protecting exposed cattle and brood sows by bacterin prophylaxis.

Outbreaks of leptospiral abortion in brood sows is best handled by combining use of bacterin with addition of 400 to 800 grams of tetracycline to the sow ration.

Encephalomyelitis

Widespread movement of horses at race tracks indicates need for protection against both western and eastern types of encephalomyelitis virus. In the past such protection has been given by injection of two doses of 2 cc. intradermal bivalent vaccine, a technic that is difficult in field practice. This past year a concentrated one cubic centimeter dose bivalent encephalomyelitis vaccine has been approved by the Agricultural Research Service.

Reports from the states of New Jersey, Pennsylvania, Massachusetts, Connecticut and Rhode Island indicate that ranched pheasants can be effectively immunized by a 1:2 dilution of encephalomyelitis vaccine in a 1 cc. subcutaneous dose.

Listeria Monocytogenes Bacterin

In 1955 a license was issued for the production of a Listeria Monocytogenes Bacterin, which has been shown to stimulate a resistance of practical significance, and has been used to some extent in sheep.

Poultry Vaccines

Our nation’s huge and rapidly expanding poultry industry requires approximately two billion doses of poultry vaccines annually. Mass immunization or so-called flock unit vaccination with a confusing list of dusts, aerosols, sprays, and water additives containing viable Newcastle and/or bronchitis viruses, are offered on an open sale basis to flock owners, except in certain states where the use of such products is restricted.

The Agricultural Research Service is currently formulating minimum requirements for:

1. Fowl pox and pigeon pox vaccine
2. Newcastle disease vaccine
3. Fowl laryngotracheitis vaccine

It is sound to state that more effective supervision and control over live virus-containing vaccines is in the best interests of our multibillion dollar poultry industry.

**Avian P.P.L.O. Diagnostic Antigen**

A license has recently been issued for the production of an antigen for the agglutination test for P.P.L.O. infection in poultry. Although the control of air-sac disease and chronic respiratory disease appears to be an unsolved problem, it is believed that the availability of this diagnostic antigen will be of some value.

**PHARMACEUTICALS**

In general, the products that may be classed as pharmaceuticals are of secondary importance to regulatory and control officials as compared with the biological products. Regulatory and control people are concerned primarily with transmissible diseases, and with diagnostic and immunizing agents rather than therapeutic products. However, they do find application where we are confronted with diseases for which we do not have practical diagnostic tests or effective immunizing agents, and where eradication is not practicable. By use of suitable therapeutic agents a degree of control can be accomplished by reducing the infection in affected herds or flocks to a point where the danger of transmission of the disease to other herds or flocks is greatly minimized.

**Antibiotics**

The antibiotics may be of considerable value in this area of disease control.

Several new antibiotics which materially broaden the indications for such therapy have been made available recently. These are:

*Penicillin V (phenoxymethyl penicillin)* Produced biosynthetically and is stable in highly acid medium, thus lending itself to peroral usage. It carries the same indications as parenteral penicillin. The dosage is expressed in the metric system and one milligram is equal to approximately 1,600 U.S.P. penicillin units.

*Novobiocin* This new antibiotic produced by *Streptomyces spheroides* is active against both gram positive and negative micro-organisms. It is of particular interest because it carries a high bacteriostatic rating against staphylococci which are insensitive or resistant to other antibiotic agents. Novobiocin, given perorally to dogs as the test animal, reaches peak blood levels within three hours and maintains therapeutic levels for eight to ten hours. Because of its low toxicity, Novobiocin should prove valuable in many selective animal disease indications, especially those due to resistant staphylococci and streptococci. The dose ranges from 30 to 50 mg. per kilo of body weight. Preliminary reports indicate Novobiocin is excellent in bovine staphylococcic mastitis.

*Nystatin* While possibly of academic interest to livestock veterinarians, Nystatin is the only peroral antibiotic highly active against fungi, notably against intestinal monilia. Further clinical observations may establish usefulness for Nystatin in
dermal mycoses, histoplasmosis and certain other specific mycotic diseases of animals and birds.

**Anthelmintic Agents**

While mentioned in our committee report last year, we can again state that piperazine salts, more notably the adipate, citrate and hexahydrate, are now widely used for treatment of ascariasis in all species of animals and birds. Piperazine salts are effective in control of swine nodular worms and reasonably effective against pinworms and strongyles of horses. Piperazine salts carry the advantages of low toxicity and effectiveness if used in drinking water or in a small amount of suitable feed.

**INSECTICIDES FOR EXTERNAL PARASITES**

In the field of external insecticides, Lindane, representing the pure gamma isomer of benzene hexachloride, continues to hold a position of preference for control of livestock ectoparasites. It has proved effective against scab mites in dilutions of 0.02 to 0.07 percent while 0.4 percent dilutions upward to tolerance limits are efficient tick killers. Lindane when added to commercial screw worm smears has materially increased their effectiveness.

Dieldrin and Diazanone are of special interest to the sheep industry as these new insecticides carry long term residual activity against common ovine ectoparasites.

The search for effective parenteral or peroral agents against external parasites of livestock continues to show promise. However, none of these agents have as yet been released for sale.

**VETERINARY BIOLOGICALS CONTROL PROGRESS**

Progress has been made in two directions toward more standard control of biological products available in the United States. In the report of your committee last year, the aims and objectives of the Veterinary Biological Licensees Association were described. Briefly, this is an organization of licensed veterinary biological producers and is now made up of representatives of twenty-six producers. For the past eighteen months, this Association has been working toward the development of proposals for establishing minimum requirements for the testing of veterinary biological products. The efforts of this group to date have been directed toward minimum requirements for the testing of virus vaccines; and proposals have been agreed upon and submitted to the Agricultural Research Service for methods of testing the following products:

- Ovine-ecthyma vaccine
- Wart vaccine
- Encephalomyelitis vaccine
- Feline-distemper vaccine
- Canine-distemper vaccine
- Infectious canine hepatitis vaccine
- Canine-distemper vaccine and Infectious canine-hepatitis vaccine
Rabies vaccine
Hog-cholera vaccine

These proposals were submitted to the Animal Inspection and Quarantine Branch of the Agricultural Research Service with the recommendation that they be required of all producers of these products on a trial, temporary basis for one year; then to be adopted, possibly with revision, as experience develops in use of these methods. The Agricultural Research Service accepted these proposals and issued them, with minor revision, as temporary requirements on February 14, 1956. The V. B. L. A. has continued its work toward the development of minimum requirements for the poultry virus vaccines and has submitted proposals on part of that group of products and are actively involved in the development of similar proposals for the other poultry vaccines not yet agreed upon. Your committee believes that this cooperative effort between the veterinary biological industry and the Animal Inspection and Quarantine Branch of the A. R. S. is constructive and in the direction of more critical and standard evaluation of the quality of veterinary biologicals available to the livestock industry.

Last year, your committee recognized the need for and recommended the establishment of an impartial veterinary control laboratory assigned to the Animal Inspection and Quarantine Branch of the Agricultural Research Service. Your committee believed that such a federal control laboratory is vitally needed for the following control functions:

1. Occasional check testing of veterinary biological products produced under licenses issued by the Department of Agriculture.

2. Development and evaluation of methods for critical and reproducible testing of veterinary biologicals produced by industry. Such methods would permit setting quality standards and then evaluating products to those standards by all industry producers.

Your committee last year presented a resolution to the Resolutions Committee of this Association for support of this plan and asked that it be referred to the Legislative Committee to follow up on the progress of this plan through Congress. The plans for such a laboratory have since been formulated and approved, as it is our understanding that these facilities are to be part of the new federal laboratory to be located at Ames, Iowa.

In regard to the planned activities of the biological products control laboratory, the committee has received the following statement from Dr. J. M. Hejl, Chief, Biological Products Licensing Section:

"It is planned to test biological products both before and after licenses are issued. Before licenses are issued products will be evaluated for safety, purity, and potency with particular emphasis placed on new products that are developed. After licenses are issued samples will be collected from every serial produced and a spot check testing system will be started. The percentage of tests to be conducted has not been definitely determined at this stage; however, licensees will not know what serial will be tested since samples from every serial will be submitted. It is planned to collect samples from the field after they have been subjected to transportation and storage conditions. This additional testing will also be an integral part of our program. One of the main purposes of the laboratory will be to improve minimum standards for the
testing of veterinary biologics and to develop newer and better methods for testing these products.

"We also plan to produce various materials which will be used as standards for testing; for example, a standardized rabies virus for challenging potency test animals, standard reference vaccines and bacterins which will be tested simultaneously along with products to be released. The products must meet the results obtained from the standards. These standard materials will be made available to all licensees for their testing and, further, will be used in our laboratories for similar purposes."

With reference to the second paragraph of the statement quoted from Dr. Hejl, your committee recommends that the United States Livestock Sanitary Association request the Animal Inspection and Quarantine Branch of the A.R.S. to recognize the desirability of international uniformity of units of measurement of activity and of reference standards; and conforming insofar as is possible to the standards promulgated as international standards by the World Health Organization.

We wish to commend the Department of Agriculture for vigorously pushing forward the development of plans for these facilities. It appears to this committee that more objective and critical evaluation of new and old veterinary biological products is in prospect for the near future.
THE EVALUATION OF THE COMPLEMENT-FIXATION TEST FOR
ANAPLASMOSIS IN FIELD CONTROL
AND ERADICATION STUDIES*

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The complement-fixation test for anaplasmosis has been established as a practical and valuable diagnostic technique (1). However, very few studies (2) have been reported in connection with using the test in field control and eradication trials. Anaplasmosis continues to be a problem in many States, and outbreaks of the disease are reported in new areas yearly. The main reservoir is the infected carrier animal, and having a method to detect all infected animals is considered the key to the problem of anaplasmosis control and eradication. Surely eradication of anaplasmosis is a pertinent and worthy objective, as there is no practical method for treatment and immunization. This report is an evaluation of progress which has been made in using the complement-fixation test in several herds in which a disease-control plan was undertaken.

METHODS

The complement-fixation test (3) for anaplasmosis was performed on serums preserved with sufficient five per cent phenol to make a final concentration of 0.5 per cent. One ounce of whole blood was collected aseptically; the clot was loosened from the sides of the container and held overnight at approximately 80°F.; and the clear serum was poured off into a vial the next day. One part of five percent phenol was added to nine parts of serum. Samples prepared in this manner provided clear serums suitable for complement-fixation testing.

The presence of anaplasmosis in the herds reported in this study was established either by identification of Anaplasma marginale microscopically in stained blood smears or by inoculation of blood into a splenectomized calf. Also splenectomized calves were inoculated with blood from animals having partial or incomplete complement-fixing reactions in order to provide information on their infectivity status.

Three general herd programs have been developed in evaluating the complement-fixation test in field control and eradication studies:

(1) The initial herd test was followed by removal of all reactors for slaughter, with retesting continued at 60-90 day intervals until two successive negative herd tests were obtained.

(2) The initial herd test was followed by segregation of all reacting animals from negative ones, with semi-annual or annual retesting of the non-reacting group.

(3) The initial test was followed by semi-annual or annual retests without re-

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moval or segregation of reactors. This plan afforded information on the extent of transmission within the herd.

The following management and sanitation practices were recommended in order to diminish the opportunities for the accidental transmission and reintroduction of anaplasmosis in herds under study:

(1) Purchased animals or replacements for the herds were made only after such animals had passed two negative tests not less than 60 days apart. During the period between the required negative tests, these animals were maintained as far as possible in a separate location from other animals in the herd.

(2) Blood samples from each animal were collected with individual sterile needles. After using, the needles were placed in a bucket or other container of water. Later, the needles were thoroughly washed and sterilized by boiling.

(3) Nose leads were disinfected, after using on each animal, in water containing an antiseptic and then rinsed in a second container of clean water.

(4) Animal vaccinations and parenteral injections made in the herd for any and all purposes were carried out by using separate sterile needles on each animal, excepting tuberculin injections, which were followed by thoroughly wiping off the intradermal needle with cotton saturated with 70 percent alcohol.

(5) Tattooing, ear notching, and similar practices were carried out with instruments which had been cleaned and disinfected after using on each animal.

(6) Castrations, spaying, and other surgical procedures were carried out with instruments which had been cleaned and disinfected after using on each animal.

(7) Dehorning techniques were followed which prevented transfer of blood from one animal to another. Thorough cleaning and disinfection of all surgical dehorning instruments was performed after using on each animal.

(8) In all practices in which possibilities existed for contamination with blood, the operator's hands were washed before proceeding to the next animal.

(9) The herd owners were advised that common carrier vehicles for dead animals were not to be allowed on the premises, and that dead animals, if not burned or buried, should be moved off the premises adjacent to a public road before loading.

RESULTS AND DISCUSSION

Herd 1—In May of 1954, anaplasmosis was diagnosed for the first time in the Hawaiian Islands. The affected animal was in a dairy herd of 129 head. The Territorial Veterinarian quarantined the immediate area, and a calf-inoculation trial confirmed the disease in the suspected case. During the next 12 months the infected and adjoining herds were placed under an experimental control program. Several factors gave credance to an anaplasmosis eradication program in the Hawaiian Islands: (1) The initial recognition of anaplasmosis in 1954 suggested a low incidence of the disease; (2) biological vectors known to be capable of transmission of anaplasmosis were not present; (3) large numbers of replacement dairy animals were acquired yearly from the States, and in many cases these were obtained from areas where the disease was known to occur. Regulations refusing entry to infected animals would thus serve to prevent further introduction of the disease.
Six splenectomized calves were inoculated with randomized pools of blood collected from all cattle in the infected herd. Two neighboring herds, consisting of a total of 86 animals, were also tested in this manner, using one test calf for each herd. This test was made before it was known that the serological test would be used. The six test calves inoculated with pooled blood from the infected herd developed anaplasmosis. The two test calves inoculated with pooled blood from the two adjoining herds did not develop anaplasmosis. Subsequently, the animals in these three herds were tested for anaplasmosis by the complement-fixation technique. It was found that serums from 25 animals in the infected herd showed positive reactions. It was also observed that blood from at least three serological reactor animals had been in each of the six pools when the calf inoculations had been made on the infected herd. The 86 animals in the two adjoining herds showed negative reactions to the complement-fixation test. The reactor animals in the infected herd were slaughtered. The results of later tests in this herd are shown in Table I. Herd replacements were made with animals that had passed two negative tests at least 60 days apart. After the second negative herd test in April 1955, blood was again collected from each animal in the herd, and three pools were made and inoculated into three splenectomized calves. None of these test animals developed anaplasmosis.

This type of control program has been extended during the last year to 89 dairy herds in the Hawaiian Islands, comprising over 14,000 animals.

The status of the program in these herds as of October 31, 1956, is as follows:

<table>
<thead>
<tr>
<th>Herd Status</th>
<th>No. Animals on Last Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 herd clean (original infected herd)</td>
<td>172</td>
</tr>
<tr>
<td>34 herds with 2 successive clean tests (no reactors)</td>
<td>3,092</td>
</tr>
<tr>
<td>18 herds with 1 clean test (no reactors)</td>
<td>1,784</td>
</tr>
<tr>
<td>12 herds with 2 successive clean tests (reactors removed)</td>
<td>1,931</td>
</tr>
<tr>
<td>10 herds with 1 clean test (reactors removed)</td>
<td>2,469</td>
</tr>
<tr>
<td>13 herds with reactors on last test</td>
<td>4,707</td>
</tr>
<tr>
<td>1 herd dissolved</td>
<td></td>
</tr>
<tr>
<td>89 herds (Total)</td>
<td>14,155 (Total)</td>
</tr>
</tbody>
</table>

As of October 31, 1956, a total of 167 reactors had been removed from these herds. The regulations requiring imported cattle to pass two negative tests 60 days apart prior to introduction into clean herds have proved very successful. The bulk of the testing in the Hawaiian Islands has been done in the Territorial Laboratory, with confirmation testing performed on positive and suspicious serums at Beltsville.

Herd 2—The beef cattle herd at the North Montana Experiment Station has been under study since December 1953. Several clinical cases of anaplasmosis occurred earlier that year. The first test revealed 19 reactors, as shown in Table II. Nine of the reactors were sold for slaughter. The remaining 10 were treated with large doses of Aureomycin, and serological tests were negative eight months fol-
TABLE I

Summary of the Anaplasmosis Test and Slaughter Program Based on the Results of the Complement-Fixation Test in Herd 1 (Hawaiian Islands)

<table>
<thead>
<tr>
<th>Date</th>
<th>No. Tested</th>
<th>Test Results</th>
<th>Disposition of Positive and Suspicious Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Suspicious</td>
</tr>
<tr>
<td>1954 July</td>
<td>129</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>1954 September</td>
<td>100</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>1954 November</td>
<td>92</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>1955 January</td>
<td>120</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>1955 April</td>
<td>117</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>1956 May</td>
<td>172</td>
<td>172</td>
<td>0</td>
</tr>
</tbody>
</table>

Following treatment. These animals were left in the herd. Annual retests have revealed one new suspect in 1954 and three more suspects in 1955. No clinical cases of anaplasmosis have occurred since 1953. These animals were pastured adjacent to the Rocky Boy Indian Reservation. A test in 1955 on 1,549 cattle from the reservation indicated 177 reactors (11.4 percent). It is of interest that the experiment station herd has had so little increase in the number of serological reactors even though it is bounded by animals having a considerably higher incidence. The station herd was tested again this year and only one suspect was found.

Herd 3—A herd of beef cattle in northern Virginia has followed a reactor segregation program for three years. There were several clinical cases of anaplasmosis recognized in the herd prior to the first test in 1953. Results of the annual testing are shown in Table III. The high incidence forced the owner to maintain a reactor and a non-reactor herd. Reactors were removed for slaughter as rapidly as

TABLE II

Summary of the Anaplasmosis Slaughter and Treatment Program Based on the Results of the Complement-Fixation Test in Herd 2 (Montana)

<table>
<thead>
<tr>
<th>Date</th>
<th>No. Tested</th>
<th>Test Results</th>
<th>Disposition of Positive and Suspicious Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Suspicious</td>
</tr>
<tr>
<td>1953 December</td>
<td>333</td>
<td>314</td>
<td>0</td>
</tr>
<tr>
<td>1954 December</td>
<td>331</td>
<td>330</td>
<td>1</td>
</tr>
<tr>
<td>1955 December</td>
<td>425</td>
<td>421</td>
<td>4</td>
</tr>
</tbody>
</table>
TABLE III
Summary of Anaplasmosis Segregation Program Based on the Results of the Complement-Fixation Test in Herd 3 (Virginia)

<table>
<thead>
<tr>
<th>Date</th>
<th>No. Tested</th>
<th>Test Results</th>
<th>Disposition of Positive and Suspicious Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Suspicious</td>
</tr>
<tr>
<td>1953 April</td>
<td>164</td>
<td>103</td>
<td>14</td>
</tr>
<tr>
<td>1954 April</td>
<td>95</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>1955 April</td>
<td>109</td>
<td>107</td>
<td>2</td>
</tr>
<tr>
<td>1956 April</td>
<td>178*</td>
<td>178</td>
<td>0</td>
</tr>
</tbody>
</table>

* Includes calves.

possible without severely disrupting the economics of the herd operation. The reactor and non-reactor groups were kept in adjoining fields during the grazing season. All cattle on the farm were sprayed once every four weeks during the fly season. Annual retests were made only on those animals which had given negative reactions to the previous tests. New reactors were placed in the anaplasmosis herd. The heifer calves weaned from the reactor cows were placed with those from the non-reactors. During the winter the reactor and non-reactor herds were maintained together in a common feeding barn. The fact that no reactors were found as a result of the 1956 complement-fixation test provides encouraging evidence that this program effectively eliminated anaplasmosis in this herd. It was not possible to evaluate what role insecticides played. Combining the reactors and non-reactors during the winter months was the only practical procedure on this farm, although we would not recommend this as a sound practice. In addition it is recommended that the offspring of reactor cows pass two negative tests not less than 60 days apart, after removal from the infected herd and before being placed with negative animals.

Herd 4—Two annual anaplasmosis tests in the springs of 1955 and 1956 have been made in a large herd of Hereford cattle in southwest Virginia. The disease had not been previously suspected in this herd. The results of the first test in April 1955 are shown in Table IV. The animals with positive and suspicious reactions remained in the herd until late in the fall of 1955, when 14 with suspicious and 16 with positive reactions were sold. In November 1955 part of the remaining reactors were bled for calf-inoculation studies. Emphasis was given to those with suspicious or incomplete complement-fixing reactions in order to obtain information to aid in the interpretation of such reactions. The results are shown in Tables V and VI. Although none of the blood from animals which had shown suspicious serological reactions produced anaplasmosis in the test
Complement-Fixation Test for Anaplasmosis

Cows

Steers

(Yearlings)

Heifers

(1 & 2 yr.)

Calves

Unidentified

Total

Table IV
Summary of the Initial Anaplasmosis Test of Herd 4, April 1955

<table>
<thead>
<tr>
<th>Complement-Fixation Reactions</th>
<th>Cows</th>
<th>Bulls</th>
<th>Steers (Yearlings)</th>
<th>Heifers (1 &amp; 2 yr.)</th>
<th>Calves</th>
<th>Unidentified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>981</td>
<td>72</td>
<td>476</td>
<td>725</td>
<td>791</td>
<td>58</td>
<td>3,103</td>
</tr>
<tr>
<td>Suspicious</td>
<td>24</td>
<td>1</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>40*</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29†</td>
</tr>
<tr>
<td>Anticomplementary</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6‡</td>
</tr>
<tr>
<td>Total</td>
<td>1,032</td>
<td>74</td>
<td>492</td>
<td>729</td>
<td>793</td>
<td>58</td>
<td>3,178</td>
</tr>
</tbody>
</table>

* 14 sold; 21 negative on retest; 5 positive on retest.
† 16 sold; 2 died; 10 positive and 1 suspicious on retest.
‡ 5 negative on retest; 1 sold.

calves, these studies were complicated by the occurrence of heavy infections with Haemobartonella in the test animals. This condition appears to occur in practically all splenectomized calves to some extent. Five of the eight test calves inoculated with blood from the four donor animals having positive serological reactions developed anaplasmosis. It is possible that test calf 3999 (Table VI) did not show marginal bodies because of the Haemobartonella infection, as this animal showed marked resistance when challenged with a virulent inoculum.

The second herd test in April 1956 revealed eight more animals with positive reactions in addition to those remaining from the previous test. Table VII summarizes these results. The low incidence of anaplasmosis in this herd was not surprising as clinical infection had not been recognized. The small number of new reactors on the second annual test also suggests a mildly infectious form of the disease.

The animals showing positive and suspicious reactions were, together with their calves, removed from the herd and held in isolation until sold in October 1956.

Table V
Calf Inoculation Trials on Animals in Herd 4 Having Negative and Suspicious Reactions to the Complement-Fixation Test, November 1955

<table>
<thead>
<tr>
<th>No. of Donors</th>
<th>Donor Reactions</th>
<th>Test Calf No.*</th>
<th>Results (60 Days)</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>(-)</td>
<td>4072</td>
<td>Neg.</td>
<td>Susceptible</td>
</tr>
<tr>
<td>10</td>
<td>(-)</td>
<td>4049</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>(-)†</td>
<td>4000</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>(1+)</td>
<td>4081</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>(2+) and (3+)</td>
<td>3992</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* 50 ml. citrated blood from each donor inoculated subcutaneously into splenectomized calves.
† Suspicious on test 6 months earlier.
Calf inoculation trials were made with citrated blood from 20 of this group. Approximately 15 hours intervened between the collection of blood and the inoculations, as the samples had to be transported over 400 miles. To assure the transfer of sufficient viable organisms, if present, large amounts of blood were inoculated intravenously into the test calves (200 ml. from animals with positive serological reactions and 500 ml. from animals having suspicious reactions). The test calves had been splenectomized at least six weeks earlier, and the presence

### TABLE VI

**Calf Inoculation Trials on Animals in Herd 4 Having Positive Reaction to the Complement-Fixation Test, November 1866**

<table>
<thead>
<tr>
<th>Donor Cow</th>
<th>Age</th>
<th>Titer of Donor’s Serum</th>
<th>Test Calf No.</th>
<th>Dose (ml)</th>
<th>Incubation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Marginal Bodies (Days)</td>
</tr>
<tr>
<td>AE25890</td>
<td>4-yr.</td>
<td>1:5</td>
<td>4081**</td>
<td>10.0</td>
<td>Neg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4075**</td>
<td>1.0</td>
<td>Neg.</td>
</tr>
<tr>
<td>51-133</td>
<td>5-yr.</td>
<td>1:20</td>
<td>4070</td>
<td>10.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4074</td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>AD-9934</td>
<td>5-yr.</td>
<td>1:80</td>
<td>4003</td>
<td>10.0</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4008</td>
<td>1.0</td>
<td>47</td>
</tr>
<tr>
<td>AD56222</td>
<td>4-yr.</td>
<td>1:160</td>
<td>4022†</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3999†</td>
<td>1.0</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

* Inoculations made subcutaneously.
** Susceptible on 60-day challenge.
† Animal died of acute anaplasmosis 32 days after inoculation.
‡ Resistant on 60-day challenge.

### TABLE VII

**Summary of the Second Anaplasmosis Test of Herd 4, April 1866**

<table>
<thead>
<tr>
<th>Complement-Fixation Reactions</th>
<th>Cows</th>
<th>Bulls</th>
<th>Steers (Yearlings)</th>
<th>Heifers (1 &amp; 2 yr.)</th>
<th>Calves</th>
<th>Unidentified</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1,001</td>
<td>62</td>
<td>281</td>
<td>754</td>
<td>518</td>
<td>65</td>
<td>2,681</td>
</tr>
<tr>
<td>Suspicious</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2†</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Positive</td>
<td>16*</td>
<td>2*</td>
<td>0</td>
<td>0</td>
<td>2†</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Anticomplementary</td>
<td>2†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1,024</td>
<td>64</td>
<td>281</td>
<td>754</td>
<td>522</td>
<td>65</td>
<td>2,710</td>
</tr>
</tbody>
</table>

* 10 positives remaining from 1955; 8 new positives.
† Negative on retest.
TABLE VIII
Serological Reactions and Calf Inoculation Trials with Blood Collected October 30, 1956—Herd 4

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Age 1956</th>
<th>Complement-Fixation Test Results</th>
<th>Calf Inoc. Trials 10-30-56</th>
</tr>
</thead>
<tbody>
<tr>
<td>52-150</td>
<td>4-yr.</td>
<td>-</td>
<td>Positive</td>
</tr>
<tr>
<td>52-34</td>
<td>4-yr.</td>
<td>-</td>
<td>Positive</td>
</tr>
<tr>
<td>AB48516</td>
<td>9-yr.</td>
<td>4+</td>
<td>Positive</td>
</tr>
<tr>
<td>52-86</td>
<td>4-yr.</td>
<td>4+</td>
<td>Positive</td>
</tr>
<tr>
<td>AD56347</td>
<td>6-yr.</td>
<td>4+</td>
<td>Positive</td>
</tr>
<tr>
<td>52AAD0864</td>
<td>6-mo.</td>
<td>-</td>
<td>Positive</td>
</tr>
<tr>
<td>52-53</td>
<td>4-yr.</td>
<td>(-)</td>
<td>Positive</td>
</tr>
<tr>
<td>52-169</td>
<td>4-yr.</td>
<td>(-)</td>
<td>Positive</td>
</tr>
<tr>
<td>51-263</td>
<td>5-yr.</td>
<td>-</td>
<td>Positive</td>
</tr>
<tr>
<td>51-136</td>
<td>5-yr.</td>
<td>3+</td>
<td>Positive</td>
</tr>
<tr>
<td>52-248</td>
<td>4-yr.</td>
<td>4+</td>
<td>Positive</td>
</tr>
<tr>
<td>51-107</td>
<td>4-yr.</td>
<td>4+</td>
<td>Positive</td>
</tr>
<tr>
<td>2086</td>
<td>11-yr.</td>
<td>3+</td>
<td>Positive</td>
</tr>
<tr>
<td>1915</td>
<td>11-yr.</td>
<td>(-)</td>
<td>Positive*</td>
</tr>
<tr>
<td>AD91416</td>
<td>5-yr.</td>
<td>(-)</td>
<td>Positive*</td>
</tr>
<tr>
<td>52AAD0854</td>
<td>6-mo.</td>
<td>-</td>
<td>Positive*</td>
</tr>
<tr>
<td>859</td>
<td>5-yr.</td>
<td>4+</td>
<td>Positive*</td>
</tr>
<tr>
<td>AD9844</td>
<td>6-yr.</td>
<td>3+</td>
<td>Positive</td>
</tr>
<tr>
<td>AE25890</td>
<td>5-yr.</td>
<td>2+</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* These 4 test calves received 500 ml. citrated blood intravenously. The others received 200 ml. intravenously.

** 10.0 ml. citrated blood did not produce anaplasmosis in test calf.

— means not tested.

(—) indicates negative reaction.

of Haemobartonella had reached a low level by the time of inoculation. The results of these calf inoculations and the complement-fixing reactions of each donor are shown in Table VIII. It is apparent from these results that infected animals at times may show incomplete or suspicious complement-fixing reactions. Ten of the twenty animals showed positive reactions consistently. These data also demonstrate the value of more than one test in an infected herd.

SUMMARY

The complement-fixing test for anaplasmosis has been used successfully to control the disease in a dairy herd in the Hawaiian Islands. The eradication program there has been expanded during 1956, and removal of reactors for slaughter has markedly reduced the incidence. A more complete evaluation of that program can be made after another year's work. The test has also been used on three herds in different parts of the United States in an effort to con-
trol the spread of infection. The disease has been significantly reduced in a northern Montana beef herd. In this herd, half of the reactors were sold for slaughter, and the other reactors treated with Aureomycin. A small beef herd in northern Virginia has been freed of anaplasmosis by a system of segregation and disposal of reactors. A large beef herd in southwest Virginia has been tested two consecutive years, and a low incidence of infected animals was found. Previous to testing, the disease had not been recognized in this herd. There is difficulty in knowing how to interpret incomplete or suspicious serological reactions. Some animals showing such reactions have been demonstrated to be infected, whereas others appear to be showing non-specific reactions. In the latter case, the reactions usually do not persist but disappear after a few months. Additional research is needed to clarify the infectivity status of cattle having weakly positive complement-fixing reactions.

ACKNOWLEDGMENT

The authors gratefully acknowledge the cooperation and assistance of Dr. L. O. Mott, Animal Disease and Parasite Research Branch, whose encouragement and diligent support made this report possible. They also wish to acknowledge the information on the Hawaiian Anaplasmosis Program which was supplied by Dr. E. H. Willers, Territorial Veterinarian. Doctor Willers and his staff performed the calf-inoculation trials on the Hawaiian herds and collected serums from those herds. The authors are indebted to Dr. Hadleigh Marsh and his associates at Bozeman, Montana, for the data on the North Montana Experiment Station and Rocky Boy Indian Reservation herds. Appreciation is expressed to the owners of the two herds in Virginia for their cooperation in these studies.

REFERENCES

INHIBITION OF *ANAPLASMA MARGINALE* INFECTION IN CATTLE WITH OXYTETRACYCLINE HYDROCHLORIDE

JOHN F. CHRISTENSEN, PH.D., D.V.M. AND J. BOYD HARROLD

Until recently, efforts to evaluate the efficacy of chemotherapeutic agents against infection with *Anaplasma marginale* in cattle have been based on comparison of survival rates of treated and untreated animals or of animals treated at different times with different agents. Usually, these comparisons were made without regard to the possible influences on susceptibility of such factors as age, condition of animals, type of management or handling prior to treatment, environmental conditions, or possible variations of the virulence of the etiological agent.

Lotze (1) was among the first to appreciate the need for more accurate and objective criteria for judging the therapeutic value of agents. On the basis of experimental infections of 20 miscellaneous adult animals he showed that one relatively constant feature of the infection is the pattern of anaplasma body increase, and suggested that the parasiticidal action of a drug could be evaluated if administered during this period of rise. He stated that if “the rise of infection is definitely stopped or materially delayed after the administration of the drug, it may be concluded that that particular drug merits further investigation.”

Using splenectomized calves, Miller et al (2) further defined the requirements for measuring the efficacy of drugs against the infection, specifying that the ideal time for administration of the agent occurs when anaplasma bodies are beginning their rapid increase and are present in one to 10 percent of the red blood cells. They demonstrated that aureomycin® and terramycin® showed anaplasma-inhibitory qualities in splenectomized calves and in a limited number of infected adult animals when administered according to these specifications. Brock et al (3) demonstrated similar anaplasma-inhibitory qualities of another wide-spectrum antibiotic, tetracycline hydrochloride. Splenectomized calves were used in their experiment and the drug was administered on the first day that the anaplasma-body graph showed a sharp rise, this occurring between the third and fifth days after one percent of the erythrocytes contained anaplasma bodies.

The authors had an unusual opportunity during the winter of 1955–56 to test the efficacy of the antibiotic, oxytetracycline hydrochloride (*Terramycin®*), against *A. marginale* infection in a group of Hereford heifers of uniform age, origin and condition. It is the purpose of this paper to present the results of

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1 The oxytetracycline hydrochloride (*Terramycin®*) used in this experiment was provided through the courtesy of Chas. Pfizer and Co., Inc.

2 School of Veterinary Medicine, University of California, Davis, California.

The authors wish to express their appreciation to the J. J. Hollister Ranch, Santa Barbara, California for providing the cattle used in these experiments; and to Dr. Lawrence O. Mott, Head, Viral and Rickettsial Diseases Section, Animal Disease and Parasite Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, for his cooperation in the complement-fixation testing.
these experiments and to emphasize the requirements for testing chemotherapeutic action in normal, adult cattle.

PROCEDURE

Eleven purebred Hereford heifers ranging in age from two to two and a half years were obtained from a ranch in northern California, where they had been raised since birth. They were in excellent condition and weighed between 650 and 750 pounds per animal. They were transferred to Davis via truck and placed on pasture until confinement for the experiment. Prior to inoculation with infected blood all animals were subjected to pregnancy examination and bled for anaplasmosis complement-fixation test.

The heifers were divided into two groups of four animals each and one group of three animals, and each group run through the experiment separately to permit complete clinical and hematological examinations. During the experiment, each group was confined in a concrete-floored pen equipped with head stanchions. For a few days prior to inoculation with infected blood the heifers were stanchioned daily to get them accustomed to the procedure. During the experiment, they were examined clinically and bled with little or no excitement while stanchioned. The ration during the experiment consisted of good-quality alfalfa hay only. There was free access to fresh water in the pen.

Each of two groups of heifers consisted of three complement-fixation (C-F) negative animals and one C-F positive animal, while the third group contained three C-F negative heifers. Each animal was inoculated subcutaneously with five cc of blood pooled from equal amounts obtained on the same day from two known carriers of the same strain of A. marginale. Two of the three C-F negative animals in each group were injected intramuscularly twice daily at 12 hour intervals with oxytetracycline hydrochloride at the rate of 3 mg per pound weight per day for three days, starting when the blood smear examination showed anaplasma bodies in the red blood cells to be in the early period of rapid rise. One C-F negative heifer in each group was left untreated to serve as a control. The control heifers were the smallest of each lot, and presumably the youngest, hence theoretically the least susceptible of the animals on an age basis. The C-F positive animals were not injected with the drug, and were included in two of the groups to show the behavior of the infections in relation to those of the treated and control animals. Four C-F negative pregnant heifers were placed among the six oxytetracycline-treated animals. All other heifers were nonpregnant.

All animals were subjected to frequent clinical and hematological examinations for periods varying from 41 to 60 days following inoculation with infected blood, or for a sufficient length of time to carry the untreated control animals well into the convalescent stage of the disease. While complete hematological data were obtained, the packed cell volume (PCV) determination using the Wintrobe hematocrit tube was selected as the indicator of anemia in this study. Percentage of infected red blood cells, or cells containing anaplasma bodies, was determined on Giemsa-stained cover slip blood smears by averaging the counts of 100 cells from several areas of the smear.


TABLE I

Grouping of Heifers in Experiments to Determine Effect of Oxytetracycline Hydrochloride on the Pattern of Infection with A. marginale.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Identification of heifer</th>
<th>Age at time of inoculation</th>
<th>Pregnancy</th>
<th>Complement-fixation reaction</th>
<th>Period of clinical and hematological observation</th>
<th>Treatment with oxytetracycline hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>R1 2½</td>
<td>+ (Calved normally 2-11-56)</td>
<td></td>
<td>−</td>
<td>12-12-55 to 1-31-56</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>R6 2</td>
<td>−</td>
<td></td>
<td>−</td>
<td>“ “</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>B5 2</td>
<td>−</td>
<td></td>
<td>+</td>
<td>“ “</td>
<td>Untreated carrier</td>
</tr>
<tr>
<td></td>
<td>R2 2</td>
<td>−</td>
<td></td>
<td>−</td>
<td>“ “</td>
<td>Untreated control</td>
</tr>
<tr>
<td>II</td>
<td>R3 2½</td>
<td>+ (Calved normally 3-15-56)</td>
<td></td>
<td>−</td>
<td>1-26-56 to 3-15-56</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>R8 2½</td>
<td>+ (Calved normally 2-15-56)</td>
<td></td>
<td>−</td>
<td>“ “</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>B1 2</td>
<td>−</td>
<td></td>
<td>+</td>
<td>“ “</td>
<td>Untreated carrier</td>
</tr>
<tr>
<td></td>
<td>R4 2</td>
<td>−</td>
<td></td>
<td>−</td>
<td>3-26-56</td>
<td>Untreated control</td>
</tr>
<tr>
<td>III</td>
<td>R7 2</td>
<td>−</td>
<td></td>
<td>−</td>
<td>2-23-56 to 4-4-56</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>R9 2½</td>
<td>+ (Calved normally 2-7-56)</td>
<td></td>
<td>−</td>
<td>“ “</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>R10 2</td>
<td>−</td>
<td></td>
<td>−</td>
<td>4-27-56</td>
<td>Untreated control</td>
</tr>
</tbody>
</table>

The pertinent data on the heifers in the three experimental groups are given in table 1.

EXPERIMENTAL RESULTS

Group I. The four heifers in this group were inoculated with infective blood on December 12, 1955. The anaplasma body and PCV values are shown in figure 1.

Two C-F negative heifers (R1 and R6) both showed anaplasma bodies in blood smears for the first time on the 20th day after inoculation. The three-day period of oxytetracycline administration in both animals was started on the 24th day, when anaplasma bodies were present in 7.0 and 5.0 percent of the red blood cells, respectively. In both heifers anaplasma body counts were observed to level off and decline, and were back at the level of less than one percent five days after the first injection of the drug. The lowest PCV readings encountered during the course of these very mild anaplasma infections were 24.5 and 25.6, respectively. Neither animal showed febrile reaction during the period of observation, and both maintained good appetite and condition. The slight anemia developed by
Fig. 1. Graphs showing packed cell volume of erythrocytes (PCV) and percentage of erythrocytes containing anaplasma bodies (% AB) in 4 heifers each inoculated subcutaneously with 5 cc of blood from the same carrier source on December 12, 1955. T indicates the period of administration of oxytetracycline hydrochloride in the 2 treated animals.

Fig. 2. Graphs showing packed cell volume of erythrocytes (PCV) and percentage of erythrocytes containing anaplasma bodies (% AB) in 4 heifers each inoculated subcutaneously with 5 cc of blood from the same carrier source on January 26, 1956. T indicates the period of administration of oxytetracycline hydrochloride in the 2 treated animals.

Fig. 3. Graphs showing packed cell volume of erythrocytes (PCV) and percentage of erythrocytes containing anaplasma bodies (% AB) in 3 heifers each inoculated subcutaneously with 5 cc of blood from the same carrier source on February 23, 1956. T indicates the period of administration of oxytetracycline hydrochloride in the 2 treated animals.
these animals was not of sufficient magnitude to result in noticeable pallor of mucous membranes. Heifer R1, diagnosed as pregnant prior to the experiment, calved normally on February 11, 1956, or 61 days following the infective inoculation.

The untreated control animal in this group (heifer R2) developed a typical, severe case of anaplasmosis. Anaplasma bodies were detected first on the 20th day after infection, were present in small numbers until the 27th day, increased rapidly to involve 33.0 percent of the red blood cells on the 32nd day, then declined to the level of one percent or less by the 44th day. The period of acute symptoms of anaplasmosis occurred between the 34th and 38th days, inclusive, and was shown by elevated body temperature, greatly increased pulse, depression, inappetence, pallor of mucous membranes and loss of condition. The development of these acute symptoms was coincident with the drop of packed cell volume of erythrocytes to 10.0 and the period of decline of anaplasma bodies from the peak number. Steady clinical improvement and gradual red blood cell increase was noted from the 39th day, and the animal recovered.

The C-F positive, or assumed carrier, animal in this group (heifer B5) showed low anaplasma body levels from the 22nd to the 30th day following infection. The bodies increased to reach a peak of six percent on the 32nd day, then declined to low levels again by the 36th day. This mild anaplasma infection was associated with a comparably mild anemia, in which the PCV dropped to a low of 22.0 on the 36th day. This animal showed no apparent clinical disturbance during the period of observation.

Group II. The four heifers in this group were inoculated with infective blood on January 26, 1956. The PCV and anaplasma body values during the subsequent infections are shown in figure 2. The results agreed essentially with those reported for group I.

Anaplasma bodies appeared in the red blood cells of all four animals 19 to 23 days following inoculation. The two oxytetracycline-treated animals (heifers R3 and R8) both showed cessation of further anaplasma body increase, the development of only moderate anemia, and the absence of apparent clinical symptoms immediately following administration of the drug at the 3.5 and 4.6 percent anaplasma body levels, respectively. It should be noted that the progressive rise of anaplasma bodies above the one percent level occurred considerably later in heifer R8 than in any of the other heifers. Both of these heifers had been diagnosed as pregnant at the beginning of the experiment. R3 calved normally on March 22, 1956, or 56 days after inoculation with infective blood, and R8 produced a normal calf on February 15, 1956, or 20 days after inoculation with infective blood.

The untreated control animal in group II (heifer R4) did not develop distinct clinical anaplasmosis. However, the anaplasma bodies at the peak were present in 16.0 percent of the red blood cells and the PCV at the low point was 17.5, these values being significantly greater than those shown by either treated animal. This heifer showed inappetence and indifference for one day at the peak of anaplasma body population, but no febrile reaction was noted.

The C-F positive, or assumed carrier, animal in this group (heifer B1) showed
essentially the same infection and blood patterns as the carrier animal in group I and the untreated control heifer in group II, revealing anaplasma bodies in 10.0 percent of the red blood cells at the peak and a PCV of 18.0 at the low point. This animal showed no clinical reaction throughout the period of observation.

Group III. The three heifers in this group were inoculated with infective blood on February 23, 1956. The anaplasma body and PCV values are shown in figure 3.

In two heifers (R9 and R7) the three-day period of injection of oxytetracycline hydrochloride was started on the 29th day after infection, or when the anaplasma bodies were present in 12.8 and 10.8 percent of the erythrocytes, respectively. Although these levels were considerably higher than in the other treated heifers at the beginning of medication, the same interruption of further increase was noted, and anaplasma body levels had returned to less than three percent by the 36th day. The PCV readings at the low point were 21.0 and 25.5, respectively. Neither of these animals showed febrile reaction during the course of the infections, but both were somewhat depressed and showed inappetence on the 32nd day. By the 34th day these mild clinical reactions had ceased. Heifer R9 calved normally on February 3, 1956, or approximately two weeks prior to inoculation with infective blood.

The untreated, C-F negative control (heifer R10) developed typical acute anaplasmosis, similar in all respects to the reaction described for the control heifer in group I. Anaplasma bodies involved 32.4 percent of the red blood cells at the peak on the 32nd day and had returned to one percent by the 39th day. The PCV reading at the low point was 9.1, evidence of severe anemia. Symptoms of anaplasmosis were first noted on the 29th day, when the temperature reading was 105.1. By the 32nd day, when anaplasma bodies had reached the peak and the PCV was 11.4, all the symptoms of typical acute anaplasmosis were present. Clinical response in the form of normal body temperature and returning appetite and condition was first noted on the 39th day, following which there was steady clinical and PCV improvement.

INTERPRETATIONS AND CONCLUSIONS

The results of this experiment with normal two to two and a half year-old Hereford heifers appear to substantiate the observations of Miller et al (2) and Brock et al (3) on splenectomized calves that administration of certain wide-spectrum antibiotic agents during the early period of anaplasma body increase inhibits further multiplication of the bodies. The degree of anemia associated with these inhibited infections, as shown by hematocrit readings, was relatively mild in all cases, but appeared to be greater the later medication was delayed in the rising phase of anaplasma body population, as was also pointed out by Miller et al (2). From a clinical point of view, the systemic reactions associated with infections in the oxytetracycline-treated animals in the experiments reported in this paper were inapparent in four animals where medication was started when anaplasma bodies were present in 3.5, 4.6, 5.0 and 7.0 percent of the red blood cells, and were limited to slight depression and inappetence in two heifers treated when anaplasma bodies were at levels of 10.8 and 12.8 percent.
The three heifers in the treated groups that were pregnant after infective inoculation produced normal calves during the course of the infections, further indication that the physiological disturbance was relatively slight.

The interpretation of antibiotic inhibition in heifer R8, where the progressive rise of anaplasma bodies above the one percent level was greatly delayed, might be subject to criticism on the basis that an unavoidable period of one week without blood observations preceded the detection of the rise. The belief that the medication was administered in the early increase period is based on the corresponding PCV readings, the fact that the blood was morphologically normal when observations were resumed, and the fact that the animal appeared clinically normal throughout the period in question. It is believed, therefore, that the interpretation of antibiotic inhibition in this animal is valid.

In all three experiments, the infections in the untreated control animals showed uninhibited rise of anaplasma bodies to a peak and proportionately more severe anemias. Two of these control heifers developed severe anaplasmosis having the classical symptoms of the disease, while one control showed only mild clinical reaction of short duration. In each experiment, the anaplasma body patterns and clinical reactions of the controls were in marked contrast to those of the treated animals.

Two C-F positive heifers, presumably carriers, that were observed along with the treated and control animals in two of the groups showed initial appearance of anaplasma bodies and peaks of infection at approximately the same times as the control animals, but in both cases the infection peaks were lower and the clinical reactions inapparent.

The chronological similarities between the infections in these 11 heifers in incubation period and period of rise and fall of the anaplasma bodies point toward the value of knowing the C-F reaction of each animal under experiment and of standardizing as much as possible the other factors that might influence susceptibility such as age, sex, breed, nutritional state, environment, previous handling, and strain of A. marginale used to produce infection. The conclusion appears to be justified that chemotherapeutic evaluation in adult cattle becomes more accurate as the factors influencing susceptibility are made uniform. The great value of the C-F test as a means of determining susceptibility should be stressed, and would seem to be an essential of accurate experimental work on anaplasmosis.

The conclusions derived from these experiments strengthen our knowledge of the inhibitory effect of certain antibiotics on the increase of anaplasma bodies. The practical implications of these and similar observations are not entirely clear, but the suggestion is made that there should be adequate exploration of the idea of using antibiotic-controlled infections for the production of immunity in valuable susceptible animals that are to be added to cattle herds in areas where the incidence of carrier infection is very high. If subsequent studies and surveys on the use of the C-F test for detection of carrier animals should reveal that control by this means is impossible in certain endemic areas, the alternate approach of producing a 100 percent population of resistant or partially resistant carrier animals in these areas could be considered.
Results of an experiment to determine the effect of the antibiotic, oxytetracycline hydrochloride (Terramycin®), on anaplasma body increase in Hereford heifers experimentally infected with Anaplasma marginale are reported. Eleven heifers of the same origin, condition and age were injected subcutaneously with identical 5 cc doses of blood carrying the same strain of A. marginale, after first having been subjected to the complement-fixation test for latent anaplasma infection. Six C-F negative heifers injected intramuscularly with oxytetracycline hydrochloride at the rate of 3 mg per pound per day for three days, starting when anaplasma bodies were determined by blood smear examination to be in the early period of increase, all showed prompt interruption of further increase of the bodies and correspondingly mild anemias. Three untreated, C-F negative, control heifers allowed to run the full course of the infections showed contrastingly marked infections, with anaplasma bodies rising uninterruptedly to their peaks, and correspondingly more severe anemias and symptoms of anaplasmosis. Two C-F positive heifers, assumed on this basis to be carriers of latent infection, developed mild, subclinical infections. The conclusion seems justified that the antibiotic showed pronounced inhibitory effect on anaplasma body increase.

It is suggested that chemotherapeutic evaluations in anaplasma infections using adult cattle become more measurable and accurate if the complement-fixation reactions of the cattle are known, and if the cattle are of the greatest possible uniformity in age, sex, breed, condition and other variables that might influence susceptibility.

REFERENCES

REPORT OF COMMITTEE ON ANAPLASMOSIS

K. J. Peterson, Salem, Oregon, Chairman; A. E. Crouse, Olympia, Washington; D. A. Davidson, Fort Worth, Texas; L. J. Poelma, College Park, Maryland; E. H. Willers, Honolulu, Hawaii; J. A. King, Phoenix, Arizona.

Anaplasmosis was first described by Theobald Smith and Fred L. Kilborne in Bulletin No. 1, Bureau of Animal Industry, 1893. Since that time extensive research has been done on this disease, not only in the United States, but also in foreign countries, yet because of the characteristics of the causative organism, productive research has been slow and costly. In spite of our futile attempts to develop methods whereby this disease could be controlled and eradicated, anaplasmosis continued to spread until at the present time it has been diagnosed in all but a few northern states. Surveys indicate that in some areas in excess of fifty percent of the cattle are infected carriers and in some individual herds as many as ninety percent of the cattle are carriers of the disease.

Anaplasmosis is continuing to spread, but through the use of new tools such as the complement fixation test, newly developed insect sprays and repellents and the use of antibiotics, it may now be possible to control and eradicate this disease in certain areas of the United States. In other areas, however, especially those where the important vector is the tick, control and eradication methods are not yet feasible. Tick eradication from the large range area of the United States is at present impossible. No known chemical spray is effective in protecting cattle from tick infestation during the long period which they are on range. The role which wild animals play in the spread of anaplasmosis is not known and could be an important obstacle in eradication. Mule and Blacktail deer were proven infective carriers by W. H. Boynton in California. Blood samples from an antelope shot in an endemic anaplasmosis area in Oregon and sent to the laboratory by Drs. Peterson, Koger and Beagle were positive to the complement fixation test. Research should be conducted to determine the accuracy of this test on antelope and whether or not these animals are actually carriers of this disease, since these animals are very migratory and travel long distances.

The test and slaughter method of control and eradication is not feasible when large numbers of infected vectors are present, where a reservoir of infection may exist in wild life and where there exists a heavy rate of infection in the cattle population. This method may, however, be practical in areas where these conditions do not exist. The test and slaughter method is now being attempted in Hawaii where there are no known important vectors and where the disease is not as yet firmly entrenched. The following is a report on the progress of this program in Hawaii, submitted by Dr. Ernest H. Willers, Territorial Veterinarian.

“Clinical anaplasmosis was first observed in Hawaii in May of 1954. Using the complement-fixation test as developed by the United States Agricultural Research Service for the detection of carrier animals, a test and slaughter program was undertaken in the quarantine area. While this program was being pursued to successful conclusion, survey testing of other herds indicated that imported carrier
animals were present in many other dairy herds and possibly in a few beef cattle herds. Because no known important vectors of anaplasmosis were present in Hawaii, a test and slaughter program seemed feasible. Such a program was devised and instituted in cooperation with the Agricultural Research Service becoming effective November 16, 1955. Essential ingredients of the program are: 1) Use of the complement-fixation test for the detection of carrier animals; 2) Identification and slaughter of test positive reactors; 3) Payment of indemnity to owners based upon \( \frac{1}{5} \) of the difference between appraisal and salvage values, not exceeding $100.00 per animal; matched by federal funds; 4) Testing of herds (including all cattle over two months of age) at not less than 60-day intervals until two successive negative tests have been obtained; 5) Testing of blood samples collected from all cattle slaughtered in the Territory.

The initial phase of this program was directed toward the elimination of carrier animals from the dairy herds. Results of dairy herd testing conducted to October 31, 1956 are given below:

<table>
<thead>
<tr>
<th>Type Test</th>
<th>No. Herds</th>
<th>No. Cattle</th>
<th>*No Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>80</td>
<td>14,661</td>
<td>129</td>
</tr>
<tr>
<td>Retest 1</td>
<td>61</td>
<td>11,214</td>
<td>34</td>
</tr>
<tr>
<td>Retest 2</td>
<td>21</td>
<td>4,964</td>
<td>23</td>
</tr>
<tr>
<td>Retest 3</td>
<td>6</td>
<td>1,877</td>
<td>1</td>
</tr>
</tbody>
</table>

* Note: Lowest No. Reactors—1 reactor in herd of 807

Highest No. Reactors—25 reactors in herd of 819

Indemnity payments:

- No. of reactors slaughtered to October 31, 1956: 167
- Amount of indemnities paid by Territory of Hawaii: $15,126.00
- Amount of indemnities paid by Federal: $15,126.00
"Hawaiian officials have expressed the opinion that the cooperative program as it is now being conducted, and with the limited vector situation, is a practical method for the eradication of anaplasmosis in the Territory of Hawaii. No insurmountable obstacles to the successful conclusion of the campaign can be foreseen."

A number of states are now using or are contemplating on using the complement fixation test to determine the incidence of anaplasmosis within their borders. The test is also being extensively employed as an important part of many research projects. Dr. Lawrence Mott reports that the Animal Disease and Parasite Branch of the Agricultural Research Service has trained research personnel from Florida, Louisiana, Oregon, Puerto Rico, Virginia and Wyoming in the techniques of conducting this test. The Animal Disease and Parasite Branch has also cooperated and is presently cooperating with a number of states and research institutions in conducting tests on individual animals, on herds, and on making area surveys. This cooperation is greatly appreciated by the livestock industry.

Your Committee on Anaplasmosis is of the opinion that a summary of research projects which are now being conducted in the United States and those which are contemplated in the near future would be a desirable adjunct to this report. This summary will thus be available to anyone interested in research on anaplasmosis.

**SUMMARY**

1. Studies of the reliability and possible applications of the CF test:
   Territory of Hawaii, Oregon State College, University of Tennessee and State of Wyoming.

2. Studies of the incidence of infection in individual herds and in areas within the state as shown by the CF test:

3. Studies to evaluate the efficiency of different management programs in the control of anaplasmosis:
(a) Slaughter of all animals positive to the CF test.
(b) Maintenance of cattle positive to the CF test and anaplasmosis-free cattle in separate herds on the same ranch.
(c) Maintenance of anaplasmosis positive and anaplasmosis negative cattle in the same herd with no isolation:


4. Studies pertaining to the arthropod vectors, their relationship to the spread of the disease and the search for additional unknown vectors:

Oklahoma A & M College and Oregon State College.

5. Studies pertaining to vector control:

Oklahoma A & M College, Oregon State College and Texas A & M College.

6. Studies of the natural reservoir of anaplasmosis dealing with animals other than cattle that may be potential sources of infection:

Oregon State College.

7. Studies of the etiological agent including the nature of the agent, artificial cultivation, electrophoretic pattern and the histopathological picture:

University of California, University of Florida, Louisiana State University, Oregon State College, University of Pennsylvania.

8. Studies of the wide spectrum antibiotics and their influence on the pattern of infection in anaplasmosis:

University of California.

9. Studies pertaining to the use of antibiotics in the eradication of the carrier state:

Kansas State College.

10. Studies of the feasibility of preventing anaplasmosis through the use of low cost, crude antibiotics:

Kansas State College.

11. Studies in the attempt to produce immunizing products:

Louisiana State University.

12. Studies on the effect of Anaplasma ovis on experimental sheep and goats and the differentiation of this organism from Anaplasma marginale. Also the relationship between Anaplasma ovis and bluetongue virus in production of disease in sheep:

Kansas State College.

13. Studies on the possibility of adapting the causative agent to unnatural hosts:

Louisiana State University.

14. Studies on new drugs in an attempt to find more specific curative agents:

Louisiana State University, Texas A & M College.

15. Studies on the wide spectrum antibiotic in the possibility of using antibiotic-controlled infections for immunization of valuable animals purchased for replacement on ranches in areas endemic for anaplasmosis:

University of California.

16. The Animal Disease Eradication Branch research program which is directed along three main lines of investigation:

(a) Infectivity studies in cattle and also ticks—Plans are in progress to
develop cooperative research between the Entomology Research Brand and ADP to carry out investigations on methods of studying the biology of anaplasmosis in ticks and also to survey specific areas in the United States as to the presence of infected ticks.

(b) Serological studies—Research will continue on the refinement of the serological methods for application to anaplasmosis as a research tool.

(c) Antigen production studies—Production of anaplasmosis complement-fixing antigen is still a laborious, expensive, and time-consuming task. Research will continue to explore methods of antigen production in order to develop procedures for producing antigen which are superior to present methods.

Your committee suggests that the National Research Conference on Anaplasmosis, which last met in Stillwater, Oklahoma in 1953, again meet to review the progress made since that meeting and to formulate additional plans for future research.

Your committee also wishes to express its appreciation to Dr. Lawrence Mott for his aid in gathering information for this report.
The cooperative State-Federal brucellosis eradication campaign now is in its twenty-second year and rapidly approaching the time required to qualify all of the States as Modified Accredited Tuberculosis-Free Areas. While the initial incidence of bovine brucellosis was higher than tuberculosis, we cannot fully justify our delay in certifying the country on that basis alone. For many years, the brucellosis program was plagued by a variety of mistakes that required more time to correct than we like to recall.

It is interesting to note the impressive volume of official activities carried out over the first twenty-one years of the program. Records for that period show a total of 150 million blood serum agglutination tests and 26 million vaccinations. During the same time, approximately six million cattle were classified as reactors.

Actual progress made in controlling and eradicating bovine brucellosis has been both good and bad. At the beginning of the program, reactors disclosed by official testing dropped within six years from an estimated high of around 10 percent to 2.4 percent. However, in 1942 the reactor rate started climbing and by 1946 was again up to five percent. Although wartime restriction of various kinds contributed to this situation, they do not fully explain the 13 years needed to regain the status which was originally achieved within a period of six years. Perhaps we were operating more effectively during the early stages of the program than was realized at the time.

Fortunately, in most instances we were able to profit from our mistakes and did not lose as much in the long run as might have been the case otherwise. Certainly, several truths were established during this period. Outstanding among these are the following: (a) support of the program by the livestock industry is essential and can be gained only through effective informational campaigns, (b) uniform procedures must be carried out in order to avoid confusion and loss of confidence on the part of all concerned, (c) vaccination has definite limitations, as well as advantages, and must be employed in an intelligent manner and (d) area work is fundamental to consistent progress and the maintenance of gains. While there are other examples, these will suffice to make the point intended.

When we stop and realize that since 1934 brucellosis has cost the cattle industry of this country more than 2 billion dollars, the effort that is going into the eradication project becomes less imposing and more fully justified. Based on current values, it has been estimated that for each 0.5 percent reduction in the nationwide incidence of bovine brucellosis, we can expect to realize a saving of approximately 9 million dollars each year in losses caused by the disease. With about $50 million still being lost each year because of brucellosis, we can well afford to invest heavily in an effective eradication program.

Dr. C. K. Mingle, Chief, Brucellosis Eradication Section, Animal Disease Eradication Branch, Agricultural Research Service, United States Department of Agriculture.
FIRST TWO-YEAR PERIOD OF THE ACCELERATED PROGRAM

As most of you know, two years of the expanded eradication campaign now have been completed and the rapidity with which the program was accelerated following its inception in October 1954 demonstrates the high interest that exists among livestock owners generally in freeing their herds of brucellosis.

Over this period of time, funds expended on the program were significantly increased from both State and Federal sources. For fiscal years 1955 and 1956, the expenditures made in connection with the cooperative State-Federal brucellosis eradication campaign totaled $61.8 million. Of this amount, $27.0 million and $34.8 million was contributed respectively by State and Federal agencies. For the current fiscal year, it is estimated that brucellosis expenses will be shared at the rate of $15.8 million State and $23.9 million Federal. This increasing financial support being given the program by the States is most encouraging.

A. Program Activities

Far more official blood tests for brucellosis were conducted during the past two fiscal years than in any similar period of the program. The total of 2.13 million herds and 30.94 million cattle tested during this time represents increases of 57 percent in the number of herds tested and 87 percent in the number of animals tested over the previous two-year period.

For fiscal years 1955 and 1956, a total of 2.92 million herds were brucellosis ring tested. This is an increase of 82 percent over the combined brucellosis ring test totals for fiscal years 1953 and 1954.

### Table I

<table>
<thead>
<tr>
<th>Activities</th>
<th>Fiscal Years</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1953 and 1954</td>
<td>1955 and 1956</td>
</tr>
<tr>
<td><strong>Blood Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Herds Tested</td>
<td>1,356,551</td>
<td>2,139,503</td>
</tr>
<tr>
<td>No. Reactor Herds</td>
<td>204,323</td>
<td>300,096</td>
</tr>
<tr>
<td>Percent</td>
<td>(15.06)</td>
<td>(14.02)</td>
</tr>
<tr>
<td>No. Cattle Tested</td>
<td>16,862,979</td>
<td>30,940,436</td>
</tr>
<tr>
<td>No. Reactor Cattle</td>
<td>343,751</td>
<td>731,771</td>
</tr>
<tr>
<td>Percent</td>
<td>(2.03)</td>
<td>(2.36)</td>
</tr>
<tr>
<td><strong>Ring Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Herds Tested</td>
<td>1,602,535</td>
<td>2,928,479</td>
</tr>
<tr>
<td>No. Suspicious Herds</td>
<td>418,823</td>
<td>534,350</td>
</tr>
<tr>
<td>Percent</td>
<td>(26.13)</td>
<td>(18.24)</td>
</tr>
<tr>
<td><strong>Vaccinations</strong></td>
<td>7,687,250</td>
<td>9,153,932</td>
</tr>
<tr>
<td><strong>Reactors Slaughtered</strong></td>
<td>206,713</td>
<td>586,815</td>
</tr>
<tr>
<td>Percent</td>
<td>(80.13)</td>
<td>(80.19)</td>
</tr>
<tr>
<td><strong>Initial County Certifications</strong></td>
<td>14</td>
<td>177</td>
</tr>
<tr>
<td><strong>Total Certified Counties</strong></td>
<td>394</td>
<td>500</td>
</tr>
</tbody>
</table>
In comparison with diagnostic tests, official vaccinations have shown only minor increases. For the past two fiscal years, ending June 30, 1956, there were 9.15 million vaccinations reported, or an increase of only 19 percent over the previous two-year totals. We would like to believe this is an expression of more interest in actual eradication than in control alone. Such a view is supported, in part at least, by the increased percentage of reactors being removed from infected herds for slaughter. In 1954, 51.2 percent of the reactors disclosed by test were slaughtered. This figure increased to 71.1 percent in 1955 and still further to 89.2 percent in 1956.

B. Changes in Infection Rates

There has not been any spectacular reduction in the overall rate of infection disclosed by testing. Based upon blood test results alone, the percent of reactors dropped from 2.6 in fiscal year 1954 to 2.19 in fiscal year 1956. Infected herd percentages changed from 14.2 in 1954 to 13.5 in 1956. It is highly significant, however, that in spite of expanded testing in many new areas and concentration of work in a number of states on BRT suspicious herds, the percentage of blood test reactors has not increased. Moreover, if the BRT negative cattle are included in calculating the indicated degree of infection, percentages for 1956 are reduced to 1.25 for animals and 7.7 for herds.

It has been encouraging to see the drop that has occurred in the number of ring test suspicious herds found in fiscal year 1954 as compared with fiscal year 1956. For 1956, 14.8 percent of the BRT herds were suspicious. This is a reduction of 11.3 percent from the figure reported in 1954.
C. Certification of Areas

During the first two years of the accelerated program, 177 new counties were qualified as Modified Certified Brucellosis-Free Areas. This compares with 14 initial certifications made in the preceding two-year period. At the end of fiscal year 1956, there was a total of 500 certified counties throughout the country, and this number is steadily increasing. The value of consolidating our gains on the basis of establishing and maintaining certified areas cannot be overlooked. All but two States now have provisions for complete area work.

As most of you know, the entire States of Washington and Wisconsin were certified in June 1956. This makes a total of five States which have achieved the same enviable distinction and there is reason to believe this list will continue to grow at an increasing rate.

INTERSTATE MOVEMENT OF CATTLE

After six years of general discussion and three years of active effort, an interstate regulation dealing with brucellosis has been formulated. As published in the Federal Register on July 12, 1956, this regulation incorporates many of the suggestions received from interested organizations and individuals following the two previous publications made in April 1954 and October 1955. These comments and suggestions were extremely helpful in our efforts to develop an effective and practical set of requirements. It should be emphasized again that the regulation includes provisions which are considered adequate, rather than only minimum, from the standpoint of limiting the spread of bovine brucellosis.

With increasing progress being made in the cooperative State-Federal brucellosis eradication campaign, the need for strengthening control over interstate
The need for developing an economical and effective screening procedure to presumptively identify Brucella-infected range herds becomes increasingly important as the eradication program advances. The brucellosis ring test meets this requirement for dairy areas and unless a comparable method can be found for use in the range States, there is danger of the brucellosis campaign in the latter areas
being seriously retarded. Widespread interest in the development of such a procedure is evidenced by reports made by this Association, the American Veterinary Medical Association and the National Brucellosis Committee within the past several months. All of these groups have recommended that efforts be made to develop an efficient plan for screen testing range herds which can be incorporated into the Uniform Methods and Rules.

The Animal Disease Eradication Branch has undertaken cooperative investigations of various possibilities in this regard at selected centers throughout the country. This work was set up along two primary lines. One deals with the testing of cull and dry cows at auction markets and slaughtering establishments and other concentration points. The other covers testing of entire herds and the identification of cows that are going to slaughter within the immediate future. Unfortunately, the data so far assembled are inadequate for conclusive evaluation.

At the present time, ten States have submitted preliminary reports on tests conducted at auction markets and slaughtering establishments. These summaries cover a total of 17,237 cattle tested in which 1,247 reactors were disclosed. The effectiveness with which herds of origin were traced varied widely, ranging from complete failure in one instance to a high of 91 percent in another. Of the 1,247 reactors found by this method of screen testing, only 367 or 29 percent were traced to originating herds.

In the case of testing conducted on ranches, with the identification of probable culls, there were five States which made preliminary reports. A compilation of these results shows a total of 215 herds tested, of which 53 were classed as infected. Based upon the results of tests made on cull and dry cows, 43 or 81 percent of the affected herds would have been identified by this type of screening procedure alone.

While a number of factors probably contributed to tracing failures, lack of proper identification on individual animals no doubt was most important. In certain instances, more competent and better directed efforts might have located some additional herds of origin. However, a great deal has been learned about how to undertake an investigation of this kind and there is reason to believe further study will produce more significant information. This work will be continued until sufficient data have been collected to permit an accurate assessment of all possible solutions to the problem.

**BRUCELLOSIS PROGRAM INFORMATION**

At the February 1956 meeting of the National Brucellosis Committee, a Special Committee was appointed to assemble and prepare for distribution the best informational material available on brucellosis program operations. This group recently completed its assignment and packets containing sample publications and a pamphlet entitled “Suggestions For a Brucellosis Eradication Program” have been distributed to State and Federal livestock sanitary officials, State extension services, and State and National brucellosis committees. In addition, the manual will be widely distributed to other interested organizations and individuals throughout the country. Essentially, the information assembled by the Committee is based
on knowledge gained through experience in those States where programs have been most successful. It should serve a worthwhile purpose in providing suggestions that can be used by some of the States for strengthening existing programs.

A short five-minute color film on the brucellosis ring test has been completed and will be available through State film libraries within the next few weeks. This movie was designed for use either alone or as a supplement to "Triple Threat of Brucellosis." It is a brief story on how the ring test can be employed to advantage in the brucellosis eradication campaign.

GENERAL COMMENTS

The accelerated program has demonstrated conclusively that the livestock industry and the general public want brucellosis eradication carried on to a successful conclusion. We know the tools for completing the job are available and there is general agreement on how they should be applied. The only thing needed, therefore, is continuation of the eradication effort at maximum levels possible.

The momentum generated in program operations over the past two years is an extremely valuable asset and should not be dissipated. We have learned by experience that hard-won progress can be lost when pressure is relaxed. On the other hand, continued advancement of the program tends to encourage even wider participation.

The first four months of the current fiscal year shows continued increases in all phases of program operations. In comparison with the same four-month period last year, there were 74.5 percent more official blood tests conducted, 13.5 percent more official vaccinations and 57.8 percent more herds ring tested.

The immediate goal is, of course, certification of all States. However, there is danger that complacency may develop in the qualified areas. This could lead to a resurgence of infection rates and consequent loss of certifications. It is essential, therefore, that certification be considered only as an advanced step toward complete eradication. With this in mind, progress beyond the certified status can be assured.

A great deal of credit must be given the 6,871 practitioners, listed as fee-basis veterinarians, for the help they have given the accelerated campaign. Without their cooperation, it would have been impossible to expand the program to present levels. The current rate of approximately 1.5 million blood tests per month is due largely to the efforts of participating practitioners.

Finally, with the important economic and public health benefits to be derived from complete eradication of brucellosis in all susceptible livestock species, our ultimate goal should be nothing less. It can be attained if we so desire.
FURTHER STUDIES ON DURATION OF IMMUNITY TO BRUCELLOSIS INDUCED IN CALF-VACCINATED CATTLE WITH STRAIN 19 VACCINE

E. R. GOODE, Jr., D.V.M.; C. A. MANTHEI, D.V.M.; T. E. AMERault, B.S.*

INTRODUCTION

Duration of immunity to brucellosis induced in cattle vaccinated as calves with Strain 19 vaccine continues to be a controversial issue because of differences of opinions and interpretations of results obtained from differently designed experiments. No attempt will be made to review completely the literature at this time since it was covered in detail by Manthei, Mingle, and Carter (6) in their article, which is the most recent one published on the subject. Evidence presented by them strongly indicated that the immunity to brucellosis induced in cattle subcutaneously vaccinated as calves with 5 ml. of Strain 19 vaccine did not decrease with an increase in age of the animals.

This experiment was initiated in 1953 when circumstances permitted acquisition of desirable animals which could be used in obtaining additional information on duration of immunity.

MATERIAL AND METHODS

Most of the cattle used in this experiment were purebred Holsteins, with the remaining ones being Jerseys or crossbreeds.

All but 18 nonvaccinated controls and 12 of 100 vaccinated animals were accumulated from three Brucella-free herds, and originated from a single source. These animals were made available because of their low milk production or unfavorable breeding performance or reduction in number of older animals to permit replacement by younger ones. The ancestors of 12 vaccinated 2-year-olds and 18 nonvaccinated cattle also originated from the same source as those of the others; however these experimental animals were born and reared in the Brucella-free herd at the Animal Disease Station. As cattle were accumulated, they were placed in Brucella-free holding barns and yards.

The vaccinated cattle received 5 ml. of Strain 19 vaccine subcutaneously when they were six, eight, or 10 months of age. Each serial lot of vaccine used was freshly prepared from selected colonial forms of Strain 19 and contained 10 to 14 billion viable cells per ml. at the time of injection. All serial lots were found to be comparable in antigenicity and pathogenicity as determined by guinea pig-inoculation studies.

Blood-serum agglutinin titer determinations were made immediately prior to and at varying intervals after vaccination. All animals showed a typical agglutinin response to Strain 19 vaccine.

With the exception of the two-year-old heifers, the brucellosis-free status of both vaccinated and nonvaccinated cattle was determined on one or more pre-exposure

parturitions. Blood, uterine contents, and samples of colostrum from each quarter of the udder were collected for bacteriological examination. In addition, agglutinin titers were determined on blood serum and quarter samples of colostrum, and guinea pig-inoculation studies were conducted on the samples of colostrum and uterine contents.

The average gestation period of both vaccinated and nonvaccinated cattle at the time of exposure to virulent Brucella abortus was 148 days. Immediately prior to exposure, all of the cattle were removed from the holding area and placed in disease-free barns that afforded isolation of individuals in either box stalls or stanchions so spaced as to prevent contact.

Because of the limited number of animals and the ratio of pregnant and non-pregnant ones in some yearly-age groups, the cattle were grouped in the following manner:

- 2 years old—14 vaccinates and 6 nonvaccinates
- 3 and 4 years old—18 vaccinates and 10 nonvaccinates
- 5 and 6 years old—24 vaccinates and 2 nonvaccinates
- 7 and 8 years old—23 vaccinates
- 9, 10, and 11 years old—21 vaccinates

Further justification for this selective grouping is that there was a closer relationship in age of many animals of two different yearly-age classifications than of some animals within a single yearly-age classification.

Each animal was exposed on the same day by instillation of 0.05 ml. of a suspension of virulent Br. abortus strain 2308 organisms on the conjunctiva of each eye. The exposure material was prepared from a 40-hour growth of Brucella and suspended in one percent peptone solution. The Brucella suspension was divided into eight different bottles, each of which was sealed with a rubber stopper, and held at a temperature of 4°–8°C. until used for exposing animals. A different bottle of this suspension was used to expose cattle in each of the eight barns. Viability counts were made on each bottle of exposure material immediately before and after exposing the animals in each barn. Each animal received 733,000 viable organisms of strain 2308. This dosage was selected on the basis of past experiments, in which a similar number of organisms produced between 80 and 90 percent infection and 70 and 80 percent abortions in susceptible cattle.

Blood-serum agglutinin titers were determined by use of the standard tube and plate agglutination tests for brucellosis. End-point titers on all samples were determined by the tube method. Bacteriological examinations of blood were carried out in the same manner described by Manthei and Carter (5) except that trypsin-case soy broth containing sodium citrate in a final dilution of one percent was used as the liquid medium and a modified tryptose agar containing 7 percent serum was used for the solid medium.

At the time of postexposure parturitions, uterine contents (placental fluid and fetus) and colostrum samples from each quarter of the udder and blood were collected. All specimens except blood were cultured on the modified tryptose agar in an atmosphere of 10 percent CO₂ for seven days. Sections of five organs and six areas of the digestive tract were cultured from each aborted fetus.

Agglutinin titer determinations were made on each sample of blood and colos-
trum, and the blood was examined bacteriologically in the manner previously described. Each of two guinea pigs was injected intraperitoneally with 5 ml of each sample of colostrum, and each of four guinea pigs was injected subcutaneously with 0.25 ml of uterine contents. Five weeks after inoculation, guinea pigs were sacrificed for observation of gross lesions, collection of blood for agglutinin titer determinations, and collection of spleens for bacteriological studies.

All recoveries of *Brucella* were identified and typed by the methods suggested in the Second Report of the WHO/FAO Expert Committee on Brucellosis.

Cows showing evidence of infection but from which no recoveries of *Brucella* were made from the udder at the time of parturition were further examined by at least four additional weekly milk culture studies.

In terminating the experiment, all nonpregnant cattle that had shown evidence of brucellosis were sacrificed and autopsied. Twenty-seven lymph glands representing the body areas, abdominal organs, mammary gland, and the urogenital tract were collected from each animal and cultured to determine whether or not localization of *Brucella* existed. Only udders and supramammary lymph glands of cows that did not show evidence of being infected during the experiment were collected and cultured at the time of sacrifice. Blood samples were also collected at that time for sero-agglutinin titer determinations.

Data acquired concerning parturition and results of related bacteriological studies, as well as postexposure sero-agglutinin titers in each group, were examined by calculations to determine the percentage of animals having localized infection and also to determine the Index of Infection. The latter being derived from application of a method of numerically evaluating responses of cattle to virulent *Br. abortus*, which was recently introduced by Goode, Manthei, and Amerault (4).

Since a paper containing the system of numerically evaluating responses of cattle to virulent *Br. abortus* exposure is in press at the time of this presentation, it is felt that the following review of this system will be helpful.

The numerical evaluation of responses of cattle to virulent *Br. abortus* is as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pregnancy terminating with abortion or premature calf and isolation of <em>Brucella</em> from uterine contents</td>
<td>5</td>
</tr>
<tr>
<td>2. Pregnancy terminating with full-term calf and isolation of <em>Brucella</em> from uterine contents</td>
<td>4</td>
</tr>
<tr>
<td>3. Isolation of <em>Brucella</em> from udder secretion</td>
<td>3</td>
</tr>
<tr>
<td>4. Two-or-more-dilution increase of postexposure sero-agglutinin titer that persists for 90 days or longer</td>
<td>2</td>
</tr>
</tbody>
</table>

When the degree of infection in an individual is determined by use of the numerical evaluation of responses, it may vary from a minimum with a value of two to a maximum with a value of 10. An animal with the maximum degree of infection is one that aborted, had uterine and udder infection, and showed a two-or-more-dilution increase in postexposure sero-agglutinin titer that persisted for 90 days or longer. The number representing the Index of Infection is the arithmetical means of the sum of the numerical values that represent the responses of cattle.
TABLE I

Response of Various Age Groups of Cattle Exposed to Virulent Brucella Abortus

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>No. Cattle</th>
<th>Pregnant</th>
<th></th>
<th>Non-Pregnant</th>
<th>Percent Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. Cattle</td>
<td>No. Premature Parturitions</td>
<td>No. Infected</td>
<td>No. Cattle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>14</td>
<td>12</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3-4 years</td>
<td>18</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>5-6 years</td>
<td>24</td>
<td>19</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7-8 years</td>
<td>23</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>9-10-11 years</td>
<td>21</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>100</td>
<td>73</td>
<td>11</td>
<td>15</td>
<td>27</td>
</tr>
</tbody>
</table>

Nonvaccinates

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>No. Cattle</th>
<th>Pregnant</th>
<th></th>
<th>Non-Pregnant</th>
<th>Percent Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. Cattle</td>
<td>No. Premature Parturitions</td>
<td>No. Infected</td>
<td>No. Cattle</td>
</tr>
<tr>
<td>2 years</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>3 years</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4 years</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5 years</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>18</td>
<td>18</td>
<td>13</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

Av. = Average

RESULTS

The response of the various age groups of both Strain 19 calf-vaccinated and nonvaccinated cattle exposed to virulent Br. abortus is shown in Table I. Additional information on individuals in each of the following groups is also considered pertinent to a complete understanding of the tabulated results.

Two-year-old vaccinates

Virulent Br. abortus was isolated from the uterine contents of all three animals that aborted and from the udder secretions of only one. Only udder infection was demonstrated in one animal that gave birth to a full-term calf and in one that was nonpregnant.

Three- and four-year-old vaccinates

Infection was demonstrated in both the uterus and udder of one cow that aborted and in only the udder of one that had a full-term calf. Brucella infection was found in the udders of two of the eight nonpregnant cattle at the time of autopsy.
Five- and six-year-old vaccinates

The five infected animals in this age group gave birth to premature calves, and virulent Br. abortus was isolated from both the uterine contents and udder secretions of four and from only the uterine contents of one.

Seven- and eight-year-old vaccinates

Virulent Br. abortus was isolated from the uterine contents of the two infected cows which aborted and from the colostrum of only one. Although Brucella was not recovered from the milk of the other animal on repeated collections, it was isolated from the two left quarters of the udder and corresponding supramammary lymph gland at the time of autopsy.

Nine-, ten-, and eleven-year-old vaccinates

Although both of the infected cows gave birth to full-term calves, infection was demonstrated in the uterine contents of one and in the udder secretions of both.

The nonvaccinated controls will be discussed as a single group and not as individual age groups, because no significant difference in susceptibility of cattle of different ages to virulent Br. abortus was demonstrated by the authors (4) in a previous experiment. Furthermore, the numbers of such animals in each age group in this experiment were not sufficiently large to make any existing differences in susceptibility significant. Nevertheless, the total number of controls compare very favorably with the number of principals in each of the various age groups. Virulent Br. abortus was isolated from both the uterine contents and udder secretions of 11 of the 13 animals which aborted and from only the uterine contents of the remaining two. Of the two infected animals that gave birth to full-term calves, Brucella was isolated from the udder and uterus of one and from only the udder of the other.

Following exposure to virulent Br. abortus, both vaccinated and nonvaccinated animals that became infected showed an increase of two or more dilutions in sero-agglutinin titers that persisted for 90 days or longer. Although the majority of cattle that resisted infection showed increases in the postexposure sero-agglutinin titers, none of these titers increased more than two dilutions and all of them receded rapidly.

The results of postexposure blood culture studies are very significant in that virulent Br. abortus was isolated from the blood of only seven (7.0%) of the 100 vaccinated cattle and from the blood of 13 (72.2%) of the 18 nonvaccinated cattle. Furthermore, bacteremia was demonstrated in five (27.7%) of 18 vaccinated cattle that had genital or mammary infection or both, whereas no infection other than the bacteremia was demonstrated in two (2.4%) of the remaining 82 vaccinated cattle. In comparison, bacteremia was demonstrated in 12 (80%) of 15 nonvaccinated cattle that had localized infection and in one (33.3%) of three which did not have localized infection.

Comparison of acquired immunity in pregnant calf-vaccinated cattle of various ages by two methods is presented in Table II. The Index of Infection is not applicable to nonpregnant cattle, because calving performance is an integral part
TABLE II
Comparison of Acquired Immunity in Pregnant Calf-Vaccinated Cattle of Various Ages

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>No. of Cattle</th>
<th>Infected</th>
<th>Index of Infection</th>
<th>Completely Susceptible</th>
<th>Partially Resistant</th>
<th>Completely Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 years</td>
<td>12</td>
<td>33.3</td>
<td>2.3</td>
<td>8.3</td>
<td>25.0</td>
<td>66.7</td>
</tr>
<tr>
<td>3-4 years</td>
<td>10</td>
<td>20.0</td>
<td>1.5</td>
<td>10.0</td>
<td>10.0</td>
<td>80.0</td>
</tr>
<tr>
<td>5-6 years</td>
<td>19</td>
<td>26.3</td>
<td>2.5</td>
<td>21.0</td>
<td>5.3</td>
<td>73.7</td>
</tr>
<tr>
<td>7-8 years</td>
<td>17</td>
<td>11.8</td>
<td>1.0</td>
<td>5.9</td>
<td>5.9</td>
<td>88.2</td>
</tr>
<tr>
<td>9-10-11 years</td>
<td>15</td>
<td>13.3</td>
<td>.9</td>
<td>.0</td>
<td>13.3</td>
<td>86.7</td>
</tr>
</tbody>
</table>

of this rating system; therefore, they were not included. Since nonpregnant cattle were not included, it should be mentioned that the percentage of localized infection in such animals was 11.1, as compared with 20.5 in the pregnant ones.

Although the difference in acquired immunity between the various age groups of pregnant vaccinated cattle is not highly significant, the difference appeared to be greater when measured by the percentage of localized infection than by the Index of Infection. The factors responsible for this are the former method does not differentiate between degrees of resistance; consequently, its measurements are expressed in absolute immunity. The latter method, however, gives full consideration to all degrees of resistance; therefore, its measurements are expressed in relative immunity. The order of groups as determined by the percentage of localized infection is changed in four of the five groups when the Index of Infection is used for such determinations. Regardless of the method used for determining susceptibility or immunity to brucellosis in cattle vaccinated as calves with Strain 19, there is no evidence to indicate that this immunity decreases with an increase in age of the animals.

The summary of results presented in Table III does not add to information on duration of immunity in calf-vaccinated cattle, but is presented for the expressed purpose of showing the difference in susceptibility of pregnant vaccinated and nonvaccinated cattle to brucellosis. The differences expressed by the two methods used to measure susceptibility or immunity are highly significant.

TABLE III
Summary of Results

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Pregnant Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cattle</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>73</td>
</tr>
<tr>
<td>Controls</td>
<td>18</td>
</tr>
</tbody>
</table>
STUDIES ON IMMUNITY TO BRUCELLOSIS

DISCUSSION

The results of this study indicate that there is no decrease in immunity with an increase in the age of cattle vaccinated subcutaneously as calves with 5 ml. of Strain 19. These findings are further supported by the results of controlled experiments on duration of immunity reported by Manthei, Mingle, and Carter (6), as well as field studies reported by Wight (7) in this country and Buddle (2) in New Zealand.

Most of the accumulated evidence on duration of immunity emphasizes that revaccination of Strain 19 calf-vaccinated cattle is neither indicated nor beneficial. Supportive evidence of this is in the reports of Berman et al. (1) and Gilman (3) in which they showed that revaccination did not enhance the immunity induced by the primary vaccination with Strain 19 for any appreciable length of time. The exact cause of the refractiveness of Strain 19 calf-vaccinated cattle to revaccination is not known; however, it appears to be associated with the use of living agent as the primary stimulus. One could speculate that the degree of immunity induced in any individual with viable Strain 19 organisms was the maximum immune response of that individual; consequently, it does not respond to restimulation with the same stimulus.

Although our research findings appear to indicate that immunity to brucellosis increases to some extent with an increase in the age of calf-vaccinated cattle, it should be stressed that these increases are slight and not considered highly significant. Furthermore, in the absence of vaccination, recent studies (4) show that there was very little or no relationship of susceptibility to age of cattle.

The Index of Infection presents itself as being a very useful research tool in determining and comparing degrees of immunity or susceptibility of cattle to brucellosis. This numerical evaluation system, however, can be effectively applied only to data that contain the results of regularly scheduled, periodic serological and bacteriological examinations of pregnant experimental cattle. One of the responses concerned with establishing the Index of Infection that warrants particular mention is the increase of two or more dilutions in postexposure sero-agglutinin titers that persist for at least 90 days. This response correctly classified 100 percent of the animals that had localized infection, whereas the sero-agglutinin titers of animals that resisted exposure did not increase more than two dilutions and receded rapidly. The accuracy of using this response for determining the brucellosis status of cattle has been previously demonstrated by the authors. The use of this response for classifying cattle is applicable only to research, because the time of exposure is essential for making an interpretation.

Another very useful research tool is the hemaculture. When blood is cultured routinely, the incidence of bacteremia is a very good index of susceptibility of cattle to brucellosis and adds materially to interpretation of data. This has been repeatedly demonstrated in our laboratory during the past 15 years.

The information developed from this and past experiments demonstrates the undesirability of employing nonpregnant cattle for determinations of susceptibility or resistance. Because of the difficulties involved in demonstrating infection in the absence of udder secretions in open heifers and of parturition, which are reflected
in differences in the susceptibility of pregnant and nonpregnant animals, any conclusions drawn are likely to be biased. Another disadvantage of using nonpregnant cattle is that their responses cannot be applied to the formula for ascertaining the Index of Infection.

SUMMARY

The Index of Infection, which is a numerical evaluation of responses of vaccinated and nonvaccinated cattle exposed to virulent Br. abortus, more clearly shows the degree of susceptibility or immunity than does the percentage of infection.

The immunity induced in cattle subcutaneously vaccinated as calves with 5 ml. of Strain 19 vaccine did not decrease with an increase in age of the animals.

A two-or-more-dilution increase in the postexposure sero-agglutinin titer was found to be more effective than any other single criterion for classifying animals that have localized infection.

REFERENCES

THE EFFECT OF BACTERINS CONTAINING PASTEURELLA MULTOCIDA ON AGGLUTININS FOR BRUCELLA IN CATTLE*

DAVID T. BERMAN, D.V.M., PH.D.

Madison, Wisconsin

Although the serological cross reactions between the brucellae and Pasteurella multocida were described by Mallman (1) in 1930 and confirmed by Emmel and Boevers (2) and Starr and Snider (3) the existence of this cross reaction has been denied by others (4–7). It has been considered generally, even by those who recognize the existence of the cross reaction, that it does not materially influence the serological diagnosis of bovine brucellosis (3). Starr and Snider found that although the injection of commercial bacterins containing P. multocida in cattle did not result in the production of agglutinins for brucellae, a calf inoculated subcutaneously with bacterin and then with viable virulent P. multocida did develop agglutinins for brucella at diagnostically significant levels.

Several factors led to a re-examination of the problem. The early work had been done with unvaccinated cattle. It seemed not unlikely that cattle vaccinated with Strain 19 might exhibit anamnestic brucella agglutinin production when inoculated with a cross reacting antigen. Such a response has been demonstrated in rabbits inoculated with cross reacting globulin antigens (8).

In further support of this hypothesis the development of positive and suspicious titers of brucella agglutinins has been observed following the use, by practitioners, of commercial bacterins containing P. multocida in herds of Strain 19 vaccinated, brucellosis-free cattle.

The demonstration by Carter et al. (9–12) that the polysaccharide capsule of P. multocida is associated with virulence and determines serological differences among at least four serotypes furnished a method for the elucidation of the cross reactions with Br. abortus.

MATERIALS AND METHODS

Serological Tests

Tube agglutination tests employing Br. abortus antigen supplied by the Agricultural Research Service, United States Department of Agriculture were incubated at 37C for 48 hours or 56C for 18 to 24 hours. P. multocida antigens in 0.5 percent phenolized saline were adjusted to the same turbidity as the brucella antigen by means of a Coleman Nepho-Colorimeter. Agglutination tests with pasteurella antigens were incubated at 37C and the results recorded at 24 and 48 hours.

*Paper No. NS 210 from the Department of Veterinary Science, University of Wisconsin; published with the approval of the director of the Wisconsin Agricultural Experiment Station. Investigation carried out in cooperation with the Animal Disease and Parasite Research Branch, Agricultural Research Services, U. S. Dept. of Agriculture.
Cultures

Lyophilized cultures of the four serotypes of *P. multocida* and one of *P. hemolytica* were furnished by Dr. G. R. Carter, Ontario Veterinary College. The strains and their origin were as follows: type A, 50024, avian; type B, 1305-1, buffalo; type C, 211, deer; type D, W1, porcine; *P. hemolytica*, SF2, bovine.

Each culture was passed several times in mice and fluorescent colonies selected on Albini Brucella agar plates viewed with a stereoscopic dissecting microscope with obliquely transmitted light after 12 to 18 hours incubation at 37°C.

Stocks were maintained by freezing the livers, spleens, hearts and lungs of mice which had been inoculated with small numbers of organisms from a fluorescent colony.

Cell suspensions for immunization of animals or agglutination tests were prepared by streaking a few fluorescent colonies on brucella agar in petri dishes 14 cm in diameter. The colonial morphology was checked after 12 hours and the growth spread over the surface of the plates which were then incubated for another 10–12 hours. The cells were then washed from the surface of the agar with 0.25 percent formalinized saline, and the turbidity adjusted to equal that of brucella tube antigen.

Animals

Cattle inoculated with pasteurella bacterins were all Holstein-Friesian heifers maintained in a brucellosis free herd. The vaccinated animals had been inoculated as six to eight month old calves with Strain 19 three to 18 months previous to these experiments. Animals which had been inoculated with an experimental agglutinogenic brucella vaccine were included in some trials. Unvaccinated control animals were a part of each experimental group.

RESULTS

Effects of Commercial Bacterins

Seven heifers were inoculated subcutaneously with a commercial alum precipitated mixed bacterin No. 1. According to the label it contained 50 percent *P. multocida*, 30 percent *Corynebacterium* spp., 10 percent *Streptococcus* spp. and 10 percent *Micrococcus pyogenes*. Four of the animals were unvaccinated, two had been vaccinated with Strain 19 and two with the experimental vaccine. The animals received two injections of 5 and 2 ml four days apart. The brucella agglutinin response is shown in Table I. Significant changes in titer were observed only among the animals which had been previously vaccinated with brucella antigens. One of the Strain 19 vaccinates changed from a suspect to a reactor and the other became a suspect. The agglutinin titers returned to the preinoculation level by 58 days after the last inoculation of bacterin. Incubation of the tube test at 56°C for 18 to 24 hours resulted in reduction of the titers to or below the preinoculation level.

Not all of the commercial bacterins tested proved to be capable of stimulating this anamnestic response. Two different *P. multocida* preparations from different manufacturers were inactive; as was a sample of commercial mixed bacterin No. 3.

One manufacturer furnished a sample of *P. multocida* bacterin which was re-
TABLE I

The Effect of a Commercial Mixed Bacterin No. 1 on Brucella Agglutinins in Cattle

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
<th>-3</th>
<th>1</th>
<th>5</th>
<th>9</th>
<th>17</th>
<th>23</th>
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<td>I-50</td>
<td>I-50</td>
<td>I-25</td>
<td>I-25</td>
<td>I-25</td>
<td></td>
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<tr>
<td>Control</td>
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<td>I-50</td>
<td>I-50</td>
<td>I-25</td>
<td>I-25</td>
<td>I-25</td>
<td>I-50</td>
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<tr>
<td>Strain 19</td>
<td></td>
<td>I-50</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-50</td>
<td>I-50</td>
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<tr>
<td>Strain 19</td>
<td></td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
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<tr>
<td>EV*</td>
<td></td>
<td>I-50</td>
<td>I-50</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-50</td>
<td>I-50</td>
</tr>
<tr>
<td>EV</td>
<td></td>
<td>I-50</td>
<td>I-50</td>
<td>I-100</td>
<td>I-100</td>
<td>I-200</td>
<td>I-50</td>
<td>I-50</td>
</tr>
</tbody>
</table>

1 Figures are reciprocal of dilution of serum giving complete agglutination of antigen. I represents incomplete agglutination at that dilution.

2 Animals inoculated with experimental agglutinogenic vaccine.

The bacterin was not precipitated nor was it highly turbid. However, these conditions should ensure inclusion of a fairly large amount of capsular polysaccharide.

Two unvaccinated control animals, two Strain 19 vaccinates and two which had received the experimental vaccine were each given three subcutaneous inoculations of this bacterin. They received 5, 10 and 10 ml on the first, third and seventh days of the test. The brucella agglutinin response is shown in Table II. The only change in titer was seen in the Strain 19 vaccinates both of which became suspects.

TABLE II

The Effect of A Commercial Bacterin Containing P. Multocida, Types A, B and C on Brucella Agglutinins in Cattle

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
<th>-3</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 19</td>
<td></td>
<td>I-50</td>
<td>I-50</td>
<td>I-100</td>
<td>I-50</td>
<td>I-100</td>
<td>I-100</td>
<td>I-50</td>
</tr>
<tr>
<td>Strain 19</td>
<td></td>
<td>I-50</td>
<td>I-50</td>
<td>I-100</td>
<td>I-100</td>
<td>I-100</td>
<td>I-200</td>
<td>I-100</td>
</tr>
<tr>
<td>EV</td>
<td></td>
<td>I-25</td>
<td>I-25</td>
<td>I-100</td>
<td>I-100</td>
<td>I-200</td>
<td>I-25</td>
<td>I-25</td>
</tr>
</tbody>
</table>

5 ml 10 ml 10 ml

ported to contain serotypes A, B and C in the encapsulated state. According to the manufacturer, the stock strains were passed in embryonating hens' eggs until approximately 90 percent of the colonies were fluorescent. The fluorescent, encapsulated organisms were grown in a broth medium for 18 hours and the entire culture treated with formalin. The bacterin was not precipitated nor was it highly turbid. However, these conditions should ensure inclusion of a fairly large amount of capsular polysaccharide.
The Agglutinin Response of Rabbits Immunized With Monotypic Encapsulated P. Multocida Bacterins

<table>
<thead>
<tr>
<th>Type</th>
<th>Brucella Titer</th>
<th>Homologous Pasteurella Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td>C</td>
<td>320</td>
<td>640</td>
</tr>
<tr>
<td>D</td>
<td>160</td>
<td>320</td>
</tr>
</tbody>
</table>

Type Specific Bacterins

Rabbits were immunized by repeated twice weekly intravenous inoculations of each of the encapsulated and nonencapsulated “blue” strains, as well as the two types of P. hemolytica. Each rabbit received a total of 15 ml of bacterial suspension. Ten days after the last inoculation serum was obtained and the titer of agglutinins for Br. abortus and the homologous pasteurella type determined. Agglutinins for Br. abortus were observed only in the sera of animals immunized with encapsulated P. multocida cells. Types C and D cross reacted most strongly. Nonencapsulated variants and P. hemolytica did not stimulate production of agglutinins for Br. abortus. The titers for the homologous pasteurella antigens and Br. abortus are shown in Table III.

Four groups of five heifers each, three unvaccinated controls and two Strain 19 vaccinates were each inoculated with bacterins prepared from encapsulated cells of one of the four serotypes of P. multocida. The animals received 5, 10 and 12 ml on the first, third and seventh days. The brucella agglutinin response is shown in Table IV. Significant increases were seen only in animals inoculated with types C and D. For the most part this was restricted to the Strain 19 vaccinates which had received the type D bacterin, but was seen in both controls and Strain 19 vaccinates which were inoculated with type C cells. The high temperature short time tube test did not abolish these reactions. The titers returned to the preinoculation level within 35 to 60 days after the last inoculation of bacterin.

This experiment was repeated using bacterins prepared from nonencapsulated “blue” variants of P. multocida and P. hemolytica. Increases in the titer of agglutinins for Br. abortus were not seen.

Agglutinin absorption experiments indicated that encapsulated type C cells were capable of absorbing some of the agglutinins for Br. abortus from a bovine antibrucella serum. The cross reaction appeared not to be reciprocal as Br. abortus cells did not absorb agglutinins for P. multocida type C from a rabbit antitype C serum.

DISCUSSION

The data presented confirm the existence of a serological cross reaction between Br. abortus and P. multocida. This cross reaction is associated with the capsular
Table IV
The Effect of Monotypic Encapsulated P. Multocida Bacterins on Brucella Agglutinins in Cattle

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
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<tbody>
<tr>
<td></td>
<td>-25</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Strain 19</td>
<td>I-100</td>
</tr>
</tbody>
</table>

Type B Bacterin

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Strain 19</td>
<td>I-50</td>
</tr>
</tbody>
</table>

Type C Bacterin

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Strain 19</td>
<td>I-50</td>
</tr>
<tr>
<td>Strain 19</td>
<td>I-25</td>
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</table>

Type D Bacterin

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Strain 19</td>
<td>I-25</td>
</tr>
</tbody>
</table>

polysaccharides of P. multocida types C and D. The cross reaction is capable of affecting the serological diagnosis of bovine brucellosis under certain conditions.

Diagnostically significant increases in titer of agglutinins for Br. abortus, following immunization with P. multocida, are most likely to occur in animals which had previously been vaccinated with Br. abortus Strain 19. Dixon and Maurer (8) have shown that immunization with one antigen may increase the amount of antibody oriented to another antigen administered earlier if the two antigens are related. It has been demonstrated earlier in this laboratory that vaccination of
cattle with Strain 19 established a "state of preparedness" of the antibody producing tissues for the rapid production of agglutinins following revaccination (13).

The titer of agglutinins attained will also be dependent upon the residual titer of agglutinins from the vaccination with Strain 19. The fact that incubation of the agglutination test at 56°C for 24 hours did not entirely eliminate the increase in titer supports the hypothesis that the specificity of the new antibody is oriented to the determinants of Br. abortus (14, 15).

In order to stimulate the cross reacting antibody the pasteurella bacterin must contain sufficient amounts of the specific capsular polysaccharides from type C or D. The differences among commercial bacterins in their ability to produce this reaction probably lie in this requirement. Most manufacturers of bacterins adhere to the old classification of strains of P. multocida, based upon the species of animal from which they were isolated. The serological types cross these lines. Secondly, most commercial bacterins are prepared from broth cultures incubated to reach maximum turbidity. Even if the seed inoculum consists largely of encapsulated organisms prolonged cultivation in liquid medium is the best method to select for nonencapsulated mutants. It would appear that the ability of a particular lot of commercial bacterin to stimulate production of agglutinins for Br. abortus is largely a matter of chance under present manufacturing practices.

It does seem clear that immunization of previously vaccinated cattle with bacterins containing P. multocida can introduce problems in the serological diagnosis of bovine brucellosis. From the data presented it appears that a decision can be made on the significance of the agglutinins with a retest about one month after administration of the bacterin.

It remains to be seen if infections with P. multocida will also stimulate production of agglutinins in vaccinated cattle.

The cross reaction which has been demonstrated between Vibrio fetus and Br. abortus may also be found to have some effect on the serological diagnosis of bovine brucellosis. Simon (16) found agglutinins for Br. abortus in the sera of cows inoculated intravenously with viable V. fetus, but not in the sera of animals infected by way of the genital tract. Unvaccinated animals were used in these experiments. Similarly, McEntee et al. (17), and Kiggins et al. (18) concluded from work with unvaccinated animals that vibriosis should not interfere with the serological diagnosis of brucellosis. Studies should be extended to include Strain 19 vaccinated animals infected with V. fetus by the genital route.

SUMMARY

Some commercial bacterins containing Pasteurella multocida were capable of stimulating production of agglutinins for Br. abortus in cattle which had previously been vaccinated with Strain 19.

The cross reaction was associated with the capsular polysaccharide of P. multocida types C and D.

The serological and diagnostic significance of the results are discussed.

REFERENCES


REPORT OF THE SOUTHERN CONFERENCES ON BRUCELLOSIS

C. G. SCRUGGS

Dallas, Texas

Gentlemen, I am happy to tell you that by 1960 the majority of the South will be brucellosis free. There is new spirit and enthusiasm all through the South in the interest of eliminating this most costly animal disease. For instance, the State of Louisiana is testing 95 percent more cattle in this year than they did 1955. I think Louisiana is perhaps a favorable example of the tremendous progress that is being made in the South in the eradication of brucellosis.

Further, the South is going faster than any other section of the country in the job of knocking brucellosis in the head. Great progress was made in 1956 when compared even to 1955 over the whole United States. But, in the South we vaccinated 5 percent more calves than the average increase of the rest of the United States in 56. We tested 4 percent more cattle than the average increase of the rest of the United States and we slaughtered 10 percent more of our reactors than the average increase of the rest of the United States in 1956 compared to 1955. Take another set of figures, in 1953 the South tested 1,330,873 head of cattle. In 1955 the South tested 3,205,426 head of cattle. The 1956 figures I’m sure will show another substantial gain in the testing of numbers of cattle. I think all these things will illustrate to you the point I made earlier that the South is making a big progress in knocking out brucellosis and that most of the South will be modified certified brucellosis free by 1960.

I will very briefly make a rundown of the various southern states and progress they are making, the problems as reported at the recent brucellosis conferences sponsored in the south by The Progressive Farmer. By way of explanation, this year we had one conference in Atlanta, Georgia for all the states that lay on the east of the so-called southern states. We skipped a day and on the third day then we held a second brucellosis conference at Little Rock, Arkansas for the states belonging to the western half of the South, and included in that, of course, Texas and Oklahoma. Here’s the way the southern states stack up in their progress in eradicating brucellosis.

As you know, North Carolina is clear and we are sure that Doctor Rollins intends to keep North Carolina brucellosis free.

SOUTH CAROLINA: For all practical purposes, South Carolina is brucellosis free and they expect to be certified by 1957. They, for instance, have every practitioner in the state signed up and testing, they have less than one percent infection, as of now, but they have not quite tested the numbers necessary for certification.

MARYLAND: Maryland will certainly be able to come under the wire in 1957 and become modified certified free that year. They are now developing lists to check to see that they have tested all the cattle.

PUERTO RICO: We include Puerto Rico in the South because they lay very close to our Southern shores and they expect to be brucellosis free in 1957.

VIRGINIA: The state of Virginia is looking to 1958 as the year in which they
will become brucellosis free. They have an extensive program going and they have good reason to believe that they will meet the goal in 1958.

**Georgia:** Just this last week Georgia had a celebration; celebrating the fact they certified their first county as being modified certified brucellosis free in a good many years. They feel like this is the opening shot in the campaign to fully rid Georgia of brucellosis by 1960.

**Florida:** As many of you know, Florida has had extensive adult vaccination. They have plans to eliminate all use of Plan "D" very shortly. They are asking this year for a million dollars state money to get into the fight against brucellosis. They already have three counties certified. They have also developed what they call a Plan "E" which is designed purely for use in Florida where they import great numbers of cows. Because of time limitations I won't go into the details of this so-called Plan "E" but I'm sure Dr. C. L. Campbell, the state veterinarian, will be glad to explain it to you.

**West Virginia:** Their infection is less than 1 percent. They, like some other states have the problem of testing full numbers in order to be certified free. In the words of their state veterinarian, the problem now is to test the few cattle that are left "way back in the deep hollows" in West Virginia.

**Kentucky:** They have begun area work and will be ready for certification easily, they feel, by 1960.

**Alabama:** Alabama has a brucellosis committee in every county in the state and is taking big strides towards getting rid of the disease. They have begun a swine program and are taking the lead among southern states in eliminating swine brucellosis. Many other Southern states are watching their program with interest.

**Arkansas:** Arkansas has had an extensive vaccination program for eight or nine years. They, last year, vaccinated 85 percent of all the replacement heifers in Arkansas. This coming year they are asking for additional money and a compulsory vaccination program and feel that they won't have any difficulty in becoming brucellosis free by 1960.

**Mississippi:** In the last 21 months Mississippi has tested over a half million cows. They have an active program in every county. They are making some moves to control movement of cattle within the state and have, like many other states, good reason to believe that they can easily be ready by 1960.

**Tennessee:** Tennessee is moving along nicely. They have a good area testing promise. They have stepped up their testing and elimination of reactors very materially.

**Oklahoma:** Oklahoma has gotten a program underway that shows a lot of promise. They have stepped up their testing and elimination of reactors very materially. Their problem, now, is, they could probably certify a few counties but there are so many cattle gone from the counties that were there when the census was taken that they don't have the numbers to certify. That is a very real problem in many of the drouth counties. There are fewer cattle there than were when the census was taken. So it is going to be hard to certify for either brucellosis or tuberculosis because the cattle just are not there.

**Texas:** Texas has now started on an animal health program that promises great things. The livestock sanitary commission expects and fully believes that they can build the finest animal health program in the United States. As most of you know,
I am sure, they have had probably the worst in the country. Much progress has been made in bringing about understanding between various groups and segments. We expect to see great things happen in Texas because they now have a new livestock sanitary commission that fully represents all of the livestock interest in the state of Texas plus a new director and state veterinarian of the sanitary commission. Plus that, they have a very active Texas Animal Health Council, composed of the representatives of over thirty livestock and farm organizations in the state who have pledged their full cooperation to the livestock sanitary officials in cleaning up the state of Texas. As a note, you fellows in the north and other states who have been selling Texas diseased cattle, your market is about finished. In Texas we have set 1965 as a goal to be brucellosis free. We are fairly confident that we can beat that goal by three or four years.

That is a very brief rundown on the various southern states and the progress they are making. We are very happy that we are able to report such encouraging progress to you. Such was not the case three to four years ago. But thanks to the accelerated program plus the tremendous surge of interest and understanding among Southern livestock men and animal health officials we now see nothing but a brucellosis free south by 1960.

At our various brucellosis conferences there have been one or two things that are outstanding and we would like to pass them on to you for whatever they are worth. One problem that seems to have been shared by most states is that any time they ran out of funds the program suffered a tremendous set back. The big problem in the South now is not a lack of interest of eliminating brucellosis but rather a lack of people to do the testing, clerical help to do the recording of the results, and money to pay indemnities and all the necessary expenses. Based on experience of the people in the South, and I am sure experience that many of you had, the one big mistake that any state can make would be that they do not budget the money carefully enough to make it last. Be sure to always schedule your money so it will last and not run out and set you back.

One other point that stands out very starkly and very clearly to me as I have heard the reports of action in all states in the South and many others in the country is this: For any animal health control move program to succeed livestock regulatory officials must be able to control the movement of cattle within the state, at least control movements back to the farm. Controlling the movement of cattle back to the farm, to my mind, is the heart and soul of any control program. Any state that does not have legislation or authority to make sure livestock that go back to the farm and ranches are free of disease, is in for headaches in enforcing any kind of program. Unfortunately, too few states now have this authority and they badly need it. And still more discouraging is the fact that many of the states that do have the authority to control movement of cattle back to the farm do not vigorously enforce it. It is my considered judgment, if it is worth anything at all, that not until all states get strong authority to control the movement of cattle or all livestock back to the farm, they are going to have difficulty in putting into effect a good animal health program.

Gentlemen, it looks to me like that a program that has been started in Florida and a few other states on an experimental basis, promises to be one of the greatest
steps forward yet made in eradicating brucellosis in the United States. I believe that the testing of dry cows at slaughtering plants as is being done in Florida does indeed promise one of the best steps forward that we have ever made in knocking out brucellosis. It will furnish the key that will open the lock to the troublesome problem of getting the cooperation of beef cattle operators.

I would hope that you would push it with all your vigor and take all the necessary steps to work out the few troublesome details in it. I hope you will learn how, in some way, to identify cattle moving through slaughtering plants. And then when the green light is given, use the tool of testing dry cows at slaughtering plants to identify areas of infection to the fullest.

I would further suggest to you that if in some way you might change your way of thinking, as it concerns the problems of animal diseases. I would suggest to you that you begin thinking in terms of animal health, not just livestock diseases control. Take the positive approach of animal health and not the negative approach of controlling disease after it has started. We have a public health service, not a people's disease control branch. So, I second the move that's being made by some of your leaders to change the name of your type of work from livestock disease control to that of a very positive approach and call it animal health. It may not be my place to make such suggestions but if it is, I urge you to very carefully consider it. It would seem to me that it would be one of the best steps that you could take to bring about public acceptance of the kind of work you do if you would change the focus of your attention from livestock disease control to that of building a program of animal health.

I have all through the years, admired the tremendous sacrifices that many of you Control Officials have to make in terms of pay and in terms of friends among livestock producers. You are to be congratulated, for you have done a singularly fine job of keeping our country free of animal diseases. But you have largely done it alone or at least you have done it alone in far too many cases. I would further suggest to you that you call on your friends to help you to do this job. There is available too, a tremendous host of people who would like to help you eliminate animal diseases from this country. The livestock and farm organizations want to help you, colleges, the extension service, farm radio, newspaper, TV and magazine people want to cooperate with you and help rid our animals of diseases.

The Progressive Farmer is very proud of the fact that we have been able to associate with a good many of you during the last few years on this problem of brucellosis. In years gone by, The Progressive Farmer was able to help some the eradication of tick fever and tuberculosis. So, our work on brucellosis has followed right in the pattern of trying to be helpful to animal disease control officials. As many of you know, The Progressive Farmer has been allowed to sponsor five brucellosis conferences in the South since 1953. We feel like these conferences have made three worthwhile contributions.

One thing we certainly take pride in is that we may have helped all the Southern states form Brucellosis Committees. In 1953 before we held our first conference no Southern state, in so far as we knew, had a state brucellosis committee. We pushed the idea along with the very fine help of Dr. A. K. Kuttler that a state committee could be a tremendous help knocking out brucellosis. We are happy to
report to you now that all the Southern states have organized a brucellosis committee that is working or they at least have organized groups that are closely concerned with the problem of brucellosis.

We have also been pleased that we were able to originate the goal of a brucellosis free United States by 1960. The Southern states meeting at 1954 Southern brucellosis conferences first adopted the goal of a Brucellosis Free South by 1960. Since that time many other groups and many of your states have felt it was worthwhile to adopt the same goal. Since the Southern states first adopted this goal a total of 40 states now have 1960 as the date by which they hope to be modified certified free of brucellosis. Plus that, The American Farm Bureau, The National Grange, The American National Cattlemen Assn., and the National Brucellosis Committee are among some national groups who have passed strong resolutions backing the goal of 1960.

The third contribution we hope that we have been able to make is that through the medium of these conferences we have been able to get key Southern livestock leaders together to get to know one another better and to discuss many other mutual problems other than brucellosis. We have laid the ground work, I hope for many people to begin thinking in terms of animal health programs, of working out other animal disease problems that they can mutually attack. The progressive Farmer was able to do this because of the helpful cooperation of many people. But, we have no, exclusive claim on this type of thing. We urge you to contact your editor, to contact your livestock leaders and ask them for help and they can render you service much greater than we have done without too much effort. We and all like us want to help you develop and expand the work that will keep the animal health of the United States the best in the world. Call on us, we want to help you.

In closing I hope that we will have the real privilege of joining with you gentlemen in 1960 in a tremendous "Victory Over Brucellosis Celebration" somewhere in these United States.
STUDIES ON A DIFFERENTIAL TEST FOR NONSPECIFIC BRUCELLA AGGLUTINATION REACTIONS IN BOVINE SERUM*

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St. Paul, Minnesota

In a number of Northern Minnesota counties that have been certified for 15 years or more the problem of low titer sero-agglutination reactions in otherwise negative herds has been evident for many years. The introduction in the state in 1948 of a program of semi-annual milk and cream ring tests has aided in the suppression of infection in these counties to the point that the problem of herds with single reactors has come to the front. There have been instances in the past several years in which 90 percent or more of the new reactor herds, disclosed via county-wide blood tests, contained only one reactor. These reactions were not the result of calfhood vaccination, as very little calfhood vaccination with Brucella Abortus Strain 19 vaccine has been practiced in these counties.

Research efforts at the Minnesota Station have, for a number of years, been directed towards a possible solution of the above problem. In 1951, Hess and Roepke (1) reported on a filter paper chromatographic test which demonstrated that a high percentage of serums with low titers from suspect herds contained agglutinins for Brucella with properties appreciably different than those commonly present in serums with either low or high titer from reactor herds. Further reports by Hess (2,3) related to the concentration and purification of the nonspecific agglutinins for Brucella from bovine serum, and to a more detailed study of the properties of the agglutinins.

Rose (4) continued studies on the concentration and purification of the nonspecific agglutinins and their physico-chemical properties. He was able to obtain preparations that were approximately 99 percent pure and demonstrated two types of nonspecific agglutinins, as determined by differences in physico-chemical properties, such as lability to heat and isoelectric points. As a result of these studies, a rather interesting property of the nonspecific agglutinins was noted (Rose and Roepke 5), which was used as the basis of the differential test reported herein. It was found that if the serum-antigen mixture of the 1:25 dilution of the plate test was acidified with acetic or lactic acid to a pH in the region of 4, the nonspecific agglutination reactions were inhibited, whereas the specific reactions remained the same or were inhibited to a much smaller degree. It was found that

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109
suitable acidification of the serum-antigen mixture could most easily be accom-
plished by adding concentrated acetic or lactic acid to the official plate test antigen
supplied by the Agricultural Research Service, United States Department of Agri-
culture.

The results obtained with the acidified plate antigens on selected suspect serums
from suspect herds and from reactor herds were very encouraging. The studies
were then extended to the field.

The serums selected for the field studies were serums in the suspicious titer
range from what we have termed typical suspect herds, i.e., herds with one to
three suspects and no other evidence of possible infection nor any history of recent
herd infection. Serums in the reactor titer range were included, if the reactor was
the only one disclosed in the herd and when there was no other evidence of possible
infection or history of recent herd infection (typical single reactor herds).

It is the definite opinion of the authors that a differential test of the type de-
scribed in this report should be used only on serums from what we term typical
suspect herds (one to three suspects) or reactor serums from herds with one
reactor and no other evidence of infection. From a disease control and eradication
standpoint, every animal in an infected herd or one with a history of recent in-
fection should be considered a potentially dangerous animal, irrespective of its
titer to the official sero-agglutination test, until the herd is cleared by subsequent
tests.

To obtain field type information with several different acidified plate antigens
(APA) one of us was present in the trailer laboratory in Rice and Sibley counties
at the time of the original complete blood test, and again at the time of the first
retest of infected and suspect herds 60 to 120 days later. The four acidified anti-
gens used in this study are shown in Table I.

A pH change from 4.25 to 3.55 represents a five-fold (500 percent) increase in
acidity.

Two ml. amounts of the acidified antigens were prepared in one-fourth ounce
dropper bottles each day just before they were needed because the acidified anti-
gens were found to deteriorate rather rapidly. Standardized glass antigen droppers
(reduced in length to fit the dropper bottles) were used to measure the antigen for
the test. The amounts of serum and antigen used for the differential test are given
in Table II.

The first dilution represents the differential test and the last three dilutions
serve as a standard plate test control. In mixing the serum and acidified antigen

<p>| TABLE I |</p>
<table>
<thead>
<tr>
<th>Acridified Plate Test Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. Concentrated Acid Per ml. Plate Antigen</td>
</tr>
<tr>
<td>0.04 ml. Glacial (100%) Acetic</td>
</tr>
<tr>
<td>0.08 ml. Glacial (100%) Acetic</td>
</tr>
<tr>
<td>0.05 ml. Conc. (85%) lactic</td>
</tr>
<tr>
<td>0.08 ml. Conc. (85%) lactic</td>
</tr>
</tbody>
</table>
with a wire loop or toothpick the "spot size" is increased gradually to a diameter of approximately 30 mm. A thorough mixing at this point is essential to ensure a uniform acidity throughout the serum-antigen mixture. Local areas of low acidity may result in false positive reactions. The glass plate is rotated and tilted back and forth slowly after preparing the test, again after seven to eight minutes' incubation, and also after 15 minutes' incubation, to insure thorough mixing. The test is read after 15 minutes' incubation. The readings should be made with more care than is usual for the standard plate test in the field: even trace reactions should be interpreted as indicating the presence of specific agglutinins.

It is emphasized that the APA test represents an agglutination inhibition type of reaction and the agglutination frequently occurs more slowly than is usually the case for the standard plate test. Therefore all reasonable opportunity should be afforded a reaction to go to completion, in terms of time of incubation and adequate and thorough mixing. The additional care which should be used in conducting the APA test does not make it suitable for routine use on all field samples. Its use should be restricted to serums with low titers from what may be termed typical suspect herds, or on reactor serums from herds with only one reactor on which additional diagnostic information is desired. Also, as mentioned earlier, the APA test should not be used on serums from reactor herds with two or more reactors or those herds with a history of recent infection. In line with these restrictions in the use of a differential test, the data presented in this report were obtained only on serums from what we term typical suspect or single reactor herds.

The results obtained with the four acidified antigens at the time of the first area blood test in Rice and Sibley counties are summarized in Table III.

Although a high percentage of the owners shipped their suspect animals for slaughter before the 60 to 120 day retest of reactor and suspect herds, retests were obtained on 138 of the 219 suspects listed in Table III. These results are shown in Table IV.

Ten of the 12 animals (Table IV) diagnosed as reactors on the basis of the standard 60 to 120 day retests were animals on which positive APA tests had been

---

**TABLE II**

<table>
<thead>
<tr>
<th>Amounts of Serum (ml)</th>
<th>0.08</th>
<th>0.04</th>
<th>0.02</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen (0.03 ml)</td>
<td>1 Drop APA</td>
<td>1 Drop Reg. Ant.</td>
<td>1 Drop Reg. Ant.</td>
<td>1 Drop Reg. Ant.</td>
</tr>
<tr>
<td>Dilution Designation</td>
<td>1:25</td>
<td>1:50</td>
<td>1:100</td>
<td>1:200</td>
</tr>
</tbody>
</table>

---

**TABLE III**

**APA Tests on Nonvaccinated Suspects in Suspect Herds**

<table>
<thead>
<tr>
<th>No. Animals</th>
<th>APA Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4.25, %, Negative</td>
</tr>
<tr>
<td>219</td>
<td>27</td>
</tr>
</tbody>
</table>

---
TABLE IV
First 60–120 Day Retest Results on Nonvaccinated Suspects in Suspect Herds,
APA Test pH 3.55

<table>
<thead>
<tr>
<th>Original APA Test Results pH 3.55</th>
<th>60–120 Day Retest Results</th>
<th>Diagnosis (Standard Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Animals</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>104</td>
<td>98</td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
<td>70</td>
</tr>
</tbody>
</table>

observed earlier. The two animals which were negative to the APA test at pH 3.55, but diagnosed later as reactors, both showed minimum reactor titers (+1:100) and both sera were again negative to the APA test. One of the two animals was purchased for Brucella isolation studies, with negative findings. If the results of the retest are an accurate indication of possible infection in the group of 104 APA negative animals, there were no obvious errors with the APA test at pH 3.55 in this group of 104 suspect animals.

The data for the APA test at pH 3.8 were very similar to those shown in Table IV with the same distribution of the 12 reactors found on the retest. The chief difference was that the number of animals in the APA test negative group was 86 instead of 104.

It is regrettable that the results of the above studies were not available for tabulation at the time it was necessary to select a given pH for a more extensive study in the Twin City area, where the second complete area blood test was underway in a number of counties. The pH selected for the more extensive field trial was 4.0. Had the above information been available at the time, no doubt an APA test at pH 3.6 to 3.7 would have been included.

The results of the APA test at pH 4.0 on sera with low titers from nonreactor herds and reactor sera from single reactor herds are shown in Table V. Approximately five percent of the sera listed were obtained from modified certified brucellosis free counties, and slightly less than 10 percent from Rice and Sibley counties where the percent reactors found on the area test were 2.6 and 1.9 respectively. The remainder were from the Twin City area, where the level of infection was approximately 1.2 percent. The prevalence of infection in the areas from which the sera were obtained offered the varying conditions desired for a good field trial of a differential test.

Three points of major interest may be noted in Table V which are as follows:

1. The percent of negative APA tests decreases as the serum titer increases. This is due to the fact that a greater degree of inhibition of the agglutination reaction is required to change the reaction of a serum with a high titer to negative at the 1:25 dilution than is true for a serum with a low titer. (See below)

2. With the exception of the +1— titer group, the percent negative APA re-
actions in a given titer category is very nearly the same for nonvaccinated as for
calfhood-vaccinated animals. These results indicate that the agglutinins present in
the two types of serums are similar.

3. A significant percentage of the reactor serums from herds with a single
reactor and with no other evidence of possible infection were negative to the APA
test.

Table VI is a summary of the 60 to 120 day retests on 1397 of the 3418 sus-
pects listed in Table V on which APA tests were made at pH 4.0. The 21 reactor
animals in the APA negative groups will be discussed later in relation to Table
VII.

It is of interest to note the relatively low percentage of the 1397 suspect ani-
imals that were diagnosed later as reactors. If the 27 APA test positive animals

TABLE VI
60-120 Day Retest of Suspects in Suspect Herds APA Test at pH 4.0

<table>
<thead>
<tr>
<th>Type of Animals</th>
<th>Original APA Test Results pH 4.0</th>
<th>No. of Animals</th>
<th>60-120 Day Retest Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis (Standard Test)</td>
<td></td>
<td>Negative or Suspicious %</td>
</tr>
<tr>
<td>Nonvacc.</td>
<td>Negative</td>
<td>566</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>529</td>
<td>94</td>
</tr>
<tr>
<td>Vacc.*</td>
<td>Negative</td>
<td>87</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>215</td>
<td>100</td>
</tr>
</tbody>
</table>

* Original plate titers of +I- to ++I only.
diagnosed later as reactors represented an approximation of the degree of infection in the suspect animal group, the level of infection was quite low. For the non-vaccinated group, this was 2.5 percent (27 out of 1095 animals) and zero for the 302 vaccinated animals. The degree or percent of infection in a group of suspect animals from a given area would have an important bearing on the overall accuracy of a differential test, just as is true for the standard sero-agglutination test. For example, animals in low infection areas that are negative to the standard sero-agglutination test are much less likely to be infected than comparable animals in high infection areas. The same would be true for a differential test on suspect serums from suspect herds.

The detailed results of the test on the 21 APA test negative animals which were diagnosed later as reactors on the basis of the results of the standard 60 to 120 day retests are shown in Table VII. Included in the table is an animal (No. 22) which was diagnosed as negative at the time of the original test and on which a serum titer of +1:400 was obtained at the time of the retest.

With respect to the 19 nonvaccinated reactor animals listed in Table VII, there were nine instances in which the titer change in the interval between tests was what might be termed a "half dilution" or a change in titer from +I− to ++−. A high percentage of such changes is possibly due to normal inherent variations in the sero-agglutination test, as there were 91 APA test negative animals that had titers of +I−, and on which retests were obtained. There were six instances in which the titer change was a full dilution. APA retests were obtained on 16 of the 19 animals. The APA reaction changed to positive in four (25 percent) instances, the same percentage change noted for the animals diagnosed as suspects again at the time of the retest.

As indicated in Table VII, nine of the reactor animals were purchased for bacteriological studies of their tissues. Also, 11 other APA test negative animals with reactor titers at the time of the original test in other areas, were purchased for the same purpose. In addition, milk samples from 14 APA test negative reactor animals were cultured by direct culture and guinea pig inoculation of the milk sediment and cream. All attempts to isolate Brucella from the 34 APA test negative animals with reactor titers proved negative.

The changes in the APA reactions at pH 4.0 on serums from suspect animals which retained low titers in interval between blood tests are indicated in Table VIII. The percent of change in the APA reactions from negative to positive in the interval is significantly greater than the change from positive to negative. Such changes in APA reactions might present a problem in the application of a differential test of this nature, particularly with respect to animals on which blood tests are required for movement interstate or to other farms. The percentages of the changes for the APA negative as well as the APA positive groups indicated in Table VII are actually too high for suspect serums as a group, since the titers on a high percentage of the suspect serums dropped to negative at 1:50 in the interval between tests. For example, of the 566 APA test negative non-vaccinated suspects on which 60 to 120 day retests were obtained, only six percent showed positive APA reactions, a high percentage of the remainder being negative to the regular blood test.
TABLE VII
Suspects, APA Negative, in Suspect Herds Diagnosed Later as Reactors

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Original Tests</th>
<th>60-120 Day Retests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field titer. Dilutions of 50-100-200</td>
<td>APA test pH 4.0</td>
</tr>
<tr>
<td>Non-vaccinates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+---*</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>10</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>11</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>12</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>13</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>14</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>15</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>16</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>17</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>18</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>19</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>Vaccinates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>+ I</td>
<td>---</td>
</tr>
<tr>
<td>21</td>
<td>+ I--</td>
<td>---</td>
</tr>
<tr>
<td>22</td>
<td>I--</td>
<td>---</td>
</tr>
</tbody>
</table>

* = No agglutination. I = Incomplete agglutination. + = complete agglutination.
† Purchased for bacteriological studies.
‡ Not tested.

A possible explanation for the change in the APA reaction in the interval between tests may lie in the pH of the APA test used, which was 4.0. The field studies (Table III) indicate that the test at pH 4.0 does not detect an appreciable percentage of the nonspecific reactions. A slight change in the concentration or nature of the nonspecific agglutinins in the interval between tests might be sufficient in some instances to cause a change in the results of the APA test. A test at higher acidity (pH 3.75) which seems to detect an appreciably higher percentage of nonspecific reactions, might reduce somewhat the frequency of the changes in APA reactions.

In the past two months, the opportunity arose to conduct APA tests at different pH values on serums from 85 known infected animals. Fifty-one of the serums were from animals on which Brucella isolations were made as part of an-
TABLE VIII
60-120 Day APA Retests of Suspects in Suspect Herds, pH 4.0

<table>
<thead>
<tr>
<th>Type of Animal</th>
<th>Original APA Test Results pH 4.0</th>
<th>No. of Animals</th>
<th>60-120 Day APA Retest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Percent Negative</td>
</tr>
<tr>
<td>Nonvacc.</td>
<td>Negative</td>
<td>136</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>166</td>
<td>12</td>
</tr>
<tr>
<td>Vacc.*</td>
<td>Negative</td>
<td>98</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>141</td>
<td>14</td>
</tr>
</tbody>
</table>

* Original field titers of I— to ++I.

other study. Thirty-four serums were kindly supplied by Dr. D. T. Berman of the University of Wisconsin. The serum titers varied from 1:100 to 1:12,800. Portions of each of the serums were diluted with suitable amounts of pooled negative serum to result in titers of 1:200, 1:100, and 1:50 with the standard plate test. Three of the serums with standard plate test titers of 1:100 could not be included in the 1:200 group. Each of the serums in the three titer groups was then tested with the APA test at pH values from 4.0 to 2.0 with plate test antigen containing suitable amounts of acetic, lactic, tartaric or phosphoric acid. The acidity of the tests at pH 2.0 was 100 times higher than at pH 4.0.

The results of the APA tests on the serums containing the three different levels of agglutinins from known infected animals are summarized in Table IX.

Of the three serums with standard plate test titers of 1:100 two showed positive APA reactions at the 1:50 titer level to pH values as low as pH 2.50. The other serum sample showed negative APA reactions at the 1:50 titer level at pH values of 3.75 and lower and at the 1:100 titer level negative reactions at pH values of 3.50 and lower.

One rather striking point noted in the data shown in Table IX is the relationship between the acidity of the tests and the percent of positive reactions for the three levels or concentrations of specific agglutinins in the serums. When the data were plotted graphically it was noted that when the concentration of agglutinins was doubled the acidity of the APA test needed to be increased by 0.7 of a pH

TABLE IX
APA Tests on Serums from Known Infected Animals Diluted with Pooled Negative Serum to +200, +100 and +50 Plate Titers

<table>
<thead>
<tr>
<th>Serum Plate Titer</th>
<th>No. of Serums</th>
<th>pH 4.0</th>
<th>pH 3.75</th>
<th>pH 3.50</th>
<th>pH 3.25</th>
<th>pH 3.00</th>
<th>pH 2.75</th>
<th>pH 2.50</th>
<th>pH 2.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>++ +</td>
<td>82</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98.8</td>
<td>97.6</td>
<td>91.6</td>
<td></td>
</tr>
<tr>
<td>++ -</td>
<td>85</td>
<td>100</td>
<td>98.8</td>
<td>96.5</td>
<td>95.2</td>
<td>90.4</td>
<td>83.5</td>
<td>71.7</td>
<td></td>
</tr>
<tr>
<td>+ - -</td>
<td>85</td>
<td>100</td>
<td>96.5</td>
<td>91.8</td>
<td>82.3</td>
<td>71.8</td>
<td>67.8</td>
<td>64.8</td>
<td>42.5</td>
</tr>
</tbody>
</table>
unit to cause a reduction of the titer to negative at 1:25. In terms of percentages this means that an increase of 100 percent in the concentration of specific agglutinins required in increase of approximately 500 percent in acidity of the test to change the reaction to negative at the 1:25 dilution.

A very limited study of a similar nature with serums containing what appeared to be nonspecific agglutinins indicated that the relationship between acidity of the APA test and inhibition of the agglutination reaction was appreciably different than for specific agglutinins. An increase of 100 percent in the nonspecific agglutinin concentration required an increase of acidity of only 0.2 of a pH unit, or about 60 percent, to cause an inhibition of the reaction to negative at 1:25.

The data in Table IX indicate that the percent of error of the APA test on serums with low titers containing specific agglutinins is quite low in the pH range of 4.0 to 3.50. Errors of this degree cannot be determined accurately on 85 serums. The studies should be extended to obtain information on an appreciably larger number of serums to obtain more accurate estimates of the errors. The data indicate that the percent of error on serums containing low concentrations of specific agglutinins (1:50, to 1:100 titer range) is relatively small with APA tests in the pH range of 4.0 to 3.50, being of the order of 0 to 8 percent. The data very definitely indicate that as the concentration of specific agglutinins (or titer) is increased, the chances for errors decrease with an APA test, at any given pH. For example, with the APA test at pH 3.5, 91.8 percent positive tests were obtained on the serums with titers of 1:50, 98.8 percent positive reactions on serums with titers of 1:100, and 100 percent positive reactions on the serums with titers of 1:200. This feature of the APA test, of greater accuracy in detecting specific agglutinins as the titer increases, is a desirable one, because the probability of actual infection also increases as the titer increases.

If the data shown in Table IX are reasonably accurate, they suggest that an APA test at pH 3.75 might provide acceptable accuracy for a differential test on serums from non-vaccinated suspects in suspect herds or on reactor serums from herds having one reactor and no other evidence of infection.

The 60 to 120 day retests on vaccinated suspects in suspect herds (Table VI) indicate a very low level of infection in animals of this type. This finding, coupled with the fact that significant titers for vaccinated animals are one dilution higher than for non-vaccinated animals, suggests that an APA test at pH 3.50 would be satisfactory for vaccinated suspect animals in suspect herds and vaccinated reactor animals in herds with only one reactor and no other evidence of infection.

The evidence presented in this report should be considered as preliminary and not conclusive, regarding the accuracy of the APA test for specific and nonspecific agglutinins for Brucella. The evidence does suggest that the APA test may possibly have the accuracy desired for a differential test, if a proper or suitable pH or acidity of the test is selected, and if knowledge is at hand regarding the prevalence of infection in the area of origin of the serums on which additional diagnostic information is desired.

The authors wish to acknowledge the valuable contribution of the personnel in the field who are actively engaged in the brucellosis control program, for their assistance in obtaining the serums involved in the field studies, particularly to
Doctors E. H. Braunworth, D. G. DeValois, W. G. Evans, D. P. Jacobs, D. M. Murray, Alfred Peterson, G. O. Schubert, D. N. Werring, Mrs. Mary Brooke and Mrs. Marguerite Morin; also to Dr. R. L. West and the Minnesota State Livestock Sanitary Board for their cooperation in the field studies.

CONCLUSIONS

1. Field studies indicate that a high percentage of bovine serums with low titers from suspect herds contain nonspecific agglutinins for Brucella.

2. A high percentage of the nonspecific agglutination reactions for Brucella in bovine serums are inhibited if the acidity or pH of the serum-antigen mixture of the 1:25 dilution of the serum plate test is adjusted to pH 4.0 to 3.50 by the addition of suitable amounts of concentrated acetic or lactic acid to the official plate test antigen. Ninety-six to 100 percent of comparable tests on 85 serums known to contain specific agglutinins were positive or not inhibited to the extent of a negative reaction at the 1:25 dilution.

3. The preliminary field studies indicate that the acidified plate antigen (APA) test as a differential test for specific and nonspecific Brucella agglutination reactions may possess acceptable accuracy if employed only on serums with low titers from typical suspect herds or on reactor serums from herds containing one reactor and no other evidence of infection or history of recent infection.

4. The APA test would not be suitable for routine use on all serum samples, for the following reasons: 1. To secure accurate and reproducible results with the test it is necessary to use special precautions or care in conducting the test, and 2. It is not designed to be used on serums from herds with evidence of infection. Its maximum benefit would be as an aid in obtaining additional diagnostic information on selected types of serum as indicated above.

REFERENCES


REPORT OF COMMITTEE ON BRUCELLOSIS


Brucellosis in our domestic animals has been considered by this Association for a number of years. Back in the days when the eradication of tuberculosis was at its height, brucellosis was being discussed wherever regulatory officials, research men, dairy farmers or agriculture in general was being discussed. Many states during the mid-thirties inaugurated programs in cooperation with the United States Department of Agriculture whereby many thousands of herds were declared free from brucellosis and given the status as fully certified.

In the early 1940s, when vaccination of calves with Strain 19 as an adjunct to the blood test was accepted, there was every indication that this disease, costing our livestock industry millions of dollars each year, would soon be brought under control.

World War II, however, brought a halt to many of our state programs and it wasn't until 1947, at the annual meeting of this Association held in this city, that your Brucellosis Committee came out with a definite, all-inclusive program that would be workable in all areas of our country. The 1947 report received full study and discussion from both the Executive Committee of this Association and by the General Assembly, and later was given approval by the United States Department of Agriculture.

While it is true that down through the last nine years great strides have been made toward the eventual goal of complete eradication of this costly disease, yet in a program so widespread and so costly as the eradication of a major contagious and infectious disease as brucellosis, it is necessary that we appraise our work and the policies under which we are operating yearly. This has been done on several occasions, and many minor changes have been recommended by your Committee through the years. Most of these recommendations were accepted and approved by the Executive Committee and the General Assembly.

Since we met in New Orleans a year ago, Secretary Benson appointed a fact-finding committee to advise him as to whether or not the present program of brucellosis eradication was sound and, if pursued, could complete the job of eradication of the disease in the United States. That committee was made up of five able men, namely:

Dean W. A. HAGAN, Cornell University
Dean C. F. CLARK, Michigan State University
Dean F. E. PRICE, Oregon State College

119
Mr. Thomas F. Arnold, American National Cattlemen's Association

Mr. W. D. Knox, Editor, Hoard's Dairyman

This committee held sectional meetings and heard testimony of regulatory officials, extension workers, dairymen, beef growers—in fact, almost everyone who was interested in the health of our livestock was given an opportunity to appear before this committee and give whatever information they wished either for or against the present program. After collecting all of this evidence gathered over a number of months, the committee made its report and recommendations to the Secretary of Agriculture. This report, of course, is available to anyone who wishes to obtain a copy from the proper source. By and large, the report gave its stamp of approval to the procedures now being followed to bring the eradication of brucellosis to a final conclusion.

All of the states are now cooperating with the Agricultural Research Service of the United States Department of Agriculture in this program, and nearly all have set for their goal the year 1960 for final certification. Two additional states have acquired the status of modified certified brucellosis-free areas, namely, Wisconsin and Washington. This brings the total number of modified certified states up to five, and from all reports it would seem that during the next four years state after state will have met the deadline that has been set for the completion of this great task.

However, no program, no matter how well organized it may be, is so perfect that it will not need amendments or changes from year to year. With the untiring work of these people engaged in scientific endeavors and research, new tests are developed from time to time. If and when such tests have been proved beyond doubt that they are sound and can help materially in the work of controlling and eradicating diseases in our domestic animals, then and only then should these tests be accepted and incorporated in our procedures.

With that thought in mind, your Committee on Brucellosis has ever been alert and has investigated every and all proposals that would aid in any way to assist us in this great task that we have undertaken. On the evenings of Monday, Tuesday and Wednesday of this week your Committee held open hearings, wherein many suggestions were presented and considered. The Committee is now ready to submit to you its recommendations.

1. Range and semi-range areas may qualify as modified certified brucellosis-free for a period of three years provided that if, after at least 80 percent of the heifer calves retained in the area annually for not less than five years are officially vaccinated (except that vaccination is not mandatory in strictly range areas where winter feeding is not practiced), and, further, that during each year of the five-year period at least five percent of the breeding cows in the area, as determined by the statistics of the Agricultural Market Service, are subjected to the blood agglutination test for brucellosis; the blood samples to be taken from cull and slaughter cattle at ranches, sale yards, or slaughtering establishments and the percentage of infection disclosed in the area does not exceed five percent of the herds and one percent of the area cattle population over six months of age (excluding steers and spayed heifers) over the five-year period. If the test of cull and slaughter cattle does not represent a screen of all herds in the area, then all herds not
screen tested shall be subjected to the blood test. All dairy herds, and all purebred beef breeding herds shall be subjected to the official blood test within the five-year period.

2. Modified certified brucellosis-free range or semi-range areas may be maintained in a certified status for additional periods of three years, provided:

(a) That at least 80 percent of the heifer calves retained in the area annually are officially vaccinated; providing that vaccination is not mandatory in strictly range areas where winter feeding is not practiced.

(b) That during each year at least five percent of the breeding cows in the area, as determined by the statistics of the Agricultural Market Service, or a total of 15 percent during a three-year period, are subjected to the agglutination test for brucellosis; the blood samples to be taken from cull and slaughter cows at ranches, sale yards or slaughtering establishments.

(c) That herds of origin of cattle reacting at a titer of complete at 1–100 are subjected to an official blood test, and are handled according to the provisions of Part IV, Section 1-C-(3).

(d) That herds found infected during the preceding certification period are blood tested at least one year following release from quarantine.

(e) That dairy herds in the area are screened semiannually by the milk ring test, with blood test of herds reacting to the milk ring test.

(f) That the percentage of infection disclosed as a result of such tests as conducted under the provisions of the above four paragraphs does not exceed five percent of the herds, and one percent of the area cattle population over six months of age (excluding steers and spayed heifers); the number of reactors used in computing the percentage to be the number accumulated over the three-year period.

3. Your Committee greatly appreciates the opportunity to have been informed as to the present status of the “Whey Plate Test”, by Dr. H. S. Cameron, University of California, Davis, and by other research workers.

It is the unanimous opinion of your Committee that before a test can or should be recognized as an official test for any use in any or all of the several states, there should be extensive research performed in several of the nation’s leading research centers, with the results from the centers disclosing the practical efficiency of the test in freeing herds from brucellosis under varying farm conditions to determine its over-all value in the nation's eradication program.

4. Your Committee has reviewed, revised and edited, section by section, the Uniform Methods and Rules for the Establishment and Maintenance of Certified Brucellosis-free herds of cattle and Modified Certified Areas as rewritten in 1953 with amendments thereto; also included in this revision are recommendations made in this report.

The Methods and Rules have been written in four parts, as follows:

Part I: Definitions.
Part II: Recommended Procedures.
Part III: Individual certified brucellosis-free herds.
Part IV: Modified certified brucellosis-free area. Part IV of the Uniform Methods and Rules needs further study and revision; however, this study should
be reserved until the Committee meets next year, when there will be many more modified certified areas with the resulting increase of available information with which recommendation may be based on.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION BRANCH

UNIFORM METHODS AND RULES FOR THE ESTABLISHMENT AND MAINTENANCE OF CERTIFIED BRUCELLOSIS-FREE HERDS OF CATTLE AND MODIFIED CERTIFIED AREAS

Part I: Definitions

"Positive" or "Reactor"
1. An official vaccinate more than thirty (30) months of age that discloses a complete agglutination reaction in the blood titer dilution of 1/200 or higher.
2. A non-vaccinated animal more than six (6) months of age that discloses a complete agglutination reaction in the blood titer dilution of 1/100 or higher.
3. An adult vaccinate that discloses a complete agglutination reaction in the blood titer dilution of 1/100 or higher.

"Suspect"
1. An official vaccinate more than thirty (30) months of age that discloses agglutination in 1/100 and less than complete in the 1/200 dilution.
2. A non-vaccinated animal more than six (6) months of age that discloses agglutination in 1/50 and less than complete agglutination in the 1/100 dilution.
3. An adult vaccinate that discloses agglutination in 1/50 and less than complete agglutination in the 1/100 dilution.

"Negative"
1. An official vaccinate more than thirty (30) months of age that discloses a reaction of not more than complete agglutination in the 1/50 dilution.
2. A non-vaccinated animal more than six (6) months of age that discloses a reaction of less than incomplete agglutination in the 1/50 dilution.
3. An adult vaccinate that discloses a reaction of less than incomplete agglutination in the 1/50 dilution.

The "Herd" Test
Shall include all cattle over six (6) months of age except steers, spayed heifers and official vaccinates not more than thirty (30) months of age.

"Approved brucella biologic"
A product that is approved by and produced under license of the United States Department of Agriculture to be used in the control of brucellosis through its injection into cattle.
BRUCELLOSIS

"Official vaccinate"

A bovine animal that was administered an approved brucella biologic when not less than four (4) months or 120 days of age nor more than eight (8) months or 240 days of age under the supervision of a licensed, accredited veterinarian and such record of vaccination has been filed with the State Livestock Sanitary Authority on forms provided.

(a) Range and semi-range official vaccinate means a bovine animal of the recognized beef breeds that was administered an approved brucella biologic when not less than four (4) months or 120 days of age, nor more than twelve (12) months or 365 days of age under the supervision of a licensed, accredited veterinarian and such record of vaccination has been filed with the State Livestock Authority on forms provided.

"Adult vaccinate"

A bovine animal that was administered an approved brucella biologic when more than eight (8) months or 240 days of age under the supervision of a licensed, accredited veterinarian and such certificate of vaccination has been filed with the State Livestock Sanitary Authority on forms provided except as heretofore provided for range and semi-range cattle of the recognized beef breeds.

Identification of Vaccinated Animals

(a) Adult animals tattooed “AV” in the right ear or branded “AV” on right jaw.

(b) Calves tattooed “V” in right ear or branded “V” on right jaw.

(c) If the tattoo is used, then the “V” or “AV” shall be preceded by a numeral indicating the quarter of the year in which the vaccination was done. The “V” or “AV” shall be followed by the last digit of the year in which the vaccination was done.

(d) 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 1957 the “V” shall be placed with the open end facing backward and so on clockwise indefinitely.

"Immediate slaughter"

The delivery of animals to the slaughtering establishment within a ten (10) day period from the date the animals were either consigned for slaughter or permit issued for their consignment to slaughter. Such animals upon delivery to the slaughtering establishment shall be slaughtered as soon as practicable.

Part II: Recommended Procedures

SECTION I. Individual Herd Plans

* Plan A. Testing of cattle, permanent identification, and prompt disposal of positives, for slaughter only, with or without vaccination of calves.

* Herds which have passed three successive satisfactory milk or cream ring tests
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 years from the date retention of positives was started.

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 State Livestock Sanitary Authority.

Plan D. Testing of entire herds with vaccination of negative cattle only within ten (10) days after completion of test and permanent identification of positives. This plan to be used only in emergencies in herds where there is evidence of a rapid spread of brucellosis, and then only with the written approval of the State-Federal cooperating agencies. Whenever, the plan provided under this subsection is used, written notice that vaccination may not prevent the spread of such disease shall be given by the State and Federal agencies to the owner of the livestock.

SECTION II. Participation on Area Basis

A. Voluntary—When 65 percent 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 four plans.

B. Compulsory—When 75 percent or more of the cattle owners in an area have placed their cattle on any one or a combination of the four plans.

C. Compulsory—When 75 percent or more of the counties representing a majority of the cattle in the state sign up under any one or a combination of the four plans.

SECTION III. Supervision

The official brucellosis eradication programs shall be supervised by full-time employed State and/or Federal veterinarians.

SECTION IV. Entering Premises

Persons engaged in the brucellosis project should be authorized to enter premises to carry out eradication procedures.

SECTION V. Services to Owner

Services to owner should be made available without expense to him so long as funds for such purposes are available (owner to provide for the handling of his cattle). Provision should be made if possible to pay practicing veterinarians for brucellosis eradication services on a per head or on a per head and per farm basis.

SECTION VI. Classification of Animals

The following tables shall be used in classifying the blood titers of tested animals:

at not less than four (4) months nor more than six (6) months intervals may be considered as having met the brucellosis requirements of Plan A for Grade A milk production. (The Branch recommends that the above procedure be accepted as an alternate method of designating Plan A herds, provided that arrangements have been made for appropriate follow-up blood testing, as outlined under Paragraphs A, Section I, Part III, of all herds showing evidence of infection as a result of ring testing).
BRUCELLOSIS

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Negative | Negative | Suspect | Suspect | Suspect | Positive | Positive | Positive |

SECTION VII. Branding Positives

A permanent brand with the letter “B” (at least 2 x 2 inches) must be placed on the left jaw of all cattle classed positive. Positives must remain on premises where disclosed until a State or Federal permit has been obtained for movement to immediate slaughter where State Approved and/or Federal inspection is maintained.

SECTION VIII. Vaccine

Only vaccine approved and manufactured under license of United States Department of Agriculture, Agricultural Research Service, shall be used in any brucellosis control program.

SECTION IX. Movement of Cattle

No female cattle or breeding bulls over six months of age shall be sold and/or moved interstate except:

a. Animals consigned for immediate slaughter.

b. Those tested and found negative for brucellosis within 30 days prior to date of movement.

c. Cattle under 30 months of age which are official vaccinates.

d. When official records establish that the animal is a part of a certified brucellosis-free herd or area at the time of sale or movement.

SECTION X. Reports

All activities, such as results of agglutination tests and vaccination, must be reported promptly to State and Federal cooperating agencies.

Part III: Individual Certified Herd Plan

A herd may be placed under supervision for certification as brucellosis-free upon complying with provisions governing the testing requirements of the respective State-Federal Cooperative Program.

SECTION I. Herd Certification

A. Herd Tests shall be made at intervals of not more than sixty (60) days until all evidence of infection has been eliminated. A herd may be certified as brucellosis-
free when it has passed at least two consecutive negative tests, with the first clean test and the certifying test not less than twelve (12) months apart. Additional herd tests may be conducted on herds if the owner so desires, or if the certifying agency deems it advisable.

B. If there is no evidence of infection on the first test, a herd may be certified as brucellosis-free when it has passed one additional negative test conducted not earlier than six (6) months from the date of the first test.

C. Where the Milk Ring Test is employed, herds may be initially certified as brucellosis-free with a minimum of three satisfactory milk tests conducted at not less than ninety (90) day intervals and followed by a negative herd blood test conducted within ninety (90) days after the last negative milk ring test.

SECTION II. Herd Recertification

A. Upon evidence of a negative herd blood test at the end of one year, certification of that herd may be extended for one year.

B. If the retest of a certified herd or of animals from such a herd reveals one reactor, the herd may be recertified on the results of two negative herd tests conducted not less than sixty (60) days apart, with the first test at least thirty (30) days after the date of the test on which the reactor was disclosed.

C. If the retest of a certified herd or of animals from such a herd reveals more than one reactor, the herd must requalify for certification as under Section I “A”.

D. When the certified status of a herd has been cancelled only because of the presence of official vaccinates thirty (30) months of age or older disclosing continuing reaction in the dilution of 1/200 or higher, the status may be restored upon evidence of one negative herd retest applied not earlier than thirty (30) days following removal of such reactors.

E. If the retest of a certified herd discloses non-vaccinated suspects but no positives, only the suspicious animals need be retested. If all suspects are available for retest and are negative, the herd test may then be considered negative. If the retest does not include all suspicious animals, or if one or more positives result, the herd shall be classed as infected and tested as provided for in Paragraphs II “B”, II “C” or I “A”. If only one suspect was not available for retesting and if that suspect had been an official vaccinate, the herd test will be considered negative and the herd recertified.

SECTION III. General Provision

A. Vaccination titers. Official vaccinates under thirty (30) months of age are not required to be tested, or if tested, are not required to be negative. Official vaccinates over thirty (30) months of age, showing titer, may be retained in certified herds 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. To certified herds:
   a. Natural herd increase.
   b. From herds with equal status.
BRUCELLOSIS

127

c. From herds that have passed negative blood test within the past twelve (12) months.
   1. Official vaccinates under thirty (30) months of age on certificate of vaccination—over thirty (30) months of age if negative within thirty (30) days of addition, or
   2. Non-vaccinated animals on evidence of negative retest not less than sixty (60) days from date of negative herd test.

2. To herds that have passed a negative blood test within the past twelve (12) months:
   a. Natural herd increase.
   b. From herds with equal or superior status.
   c. From other herds:
      1. Official vaccinates under thirty (30) months of age on certificate of vaccination—over thirty (30) months of age if negative within thirty (30) days of addition, or
      2. Non-vaccinated animals if tested negative within thirty (30) days of addition then segregated and retested negative in not less than sixty (60) days from date of first test.

C. Additions from herds without equal status. Under qualifying conditions of Subsections B 1c and B 2c such animals shall not receive new herd status for sale purposes until they have been members at least thirty (30) days and included in a complete herd retest.

D. Certificates. Certified Brucellosis-Free Herd certificates which shall be valid for one year unless revoked, may be issued by cooperating State or Federal officials.

E. Quarantine. Animals must be confined to the premises if reactors are disclosed by the blood test. Movement of all cattle shall be prohibited until the herd has passed a negative herd retest at least thirty (30) days following removal of reactors, except cattle consigned for immediate slaughter under permit.

F. Cleaning and disinfecting. Premises shall be cleaned and disinfected under supervision or proper direction, following removal of reactors.

Part IV: Modified Certified Area Plan

The provisions of the individual certified herd plan that relate to testing, cleaning, quarantining and disinfecting shall apply to the modified certified area plan. The extent of the area shall be determined by the cooperating State and Federal Agencies. When an area has been designated and the required percentage of herds and cattle included under any of the plans, the area should be placed under quarantine and the following rules apply:

SECTION I. Area Certification

A. If as the result of a blood test of all cattle within an area the number of positives does not exceed one percent and the herd infection does not exceed five percent, the area may be declared Modified Certified Brucellosis-Free for a period of three years. Infected herds shall be quarantined until they have passed at least two consecutive negative blood tests not less than 60 days apart.
B. An area may be declared Modified Certified Brucellosis-Free by the application of two milk tests not less than six months apart, together with a blood test of all milk reacting herds and such other herds as are not included in the milk test. The number of positives must not exceed one percent of the cattle and the herd infection must not exceed five percent. Infected herds shall be quarantined until they have passed at least two consecutive blood tests not less than 60 days apart.

C. (1) Range and semi-range areas may qualify as Modified Certified Brucellosis-Free for a period of three 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 years of age in each herd, the number of positives does not exceed one percent of the area cattle population over six months of age (excluding steers and spayed heifers) and five percent of the herds.

(2) Range and semi-range areas may qualify as Modified Certified Brucellosis-Free for a period of three years provided that, if, after at least 80 percent of the heifer calves retained in the area annually for not less than five years are officially vaccinated (except that vaccination is not mandatory in strictly range or semi-range areas where winter feeding is not practical) and further, that during each year of the five-year period at least five percent of the breeding cows in the area, as determined by the statistics of the Agricultural Market Service are subjected to the blood agglutination test for Brucellosis; the blood samples to be taken from cull and slaughter cows at ranches, sale yards, or slaughtering establishments and the percentage of infection disclosed in the area does not exceed five percent of the herds and 1 percent of the area cattle population over 6 months of age (excluding steers and spayed heifers) over the five-year period. If the test of cull and slaughter cattle does not represent a screen of all herds in the area, then all herds not screen tested shall be subjected to the blood test. All Dairy herds, and all purebred beef breeding herds shall be subjected to the official blood test within the five-year period.

(3) Should evidence of infection be disclosed in any of the animals required to be tested in the range or semi-range herds under provisions of Part IV, Section I, C (1) or (2), such herds shall be quarantined until the entire herd has passed at least two consecutive tests not less than 60 days apart, or one negative herd test conducted not less than 120 days from the date reactors were removed for slaughter.

D. If testing as outlined under Part IV, Section I “A”, I “B”, or I “C” reveals an infection rate of more than one percent, but not over two percent, and a retest of the infected herds applied within 120 days discloses not more than one percent animal infection in not over five percent of the herds, the area may then be certified.

E. If the test of an area as outlined under Part IV, Section I “A”, I “B” or I “C” results in more than two percent positives, or if a retest of infected herds as under Section I “D” does not qualify the area for certification, it shall be necessary to make a complete area retest.
Section II. Area Recertification

A. At the expiration of the three-year period areas certified under the provision of Part IV, Section I “A”, I “B” may be recertified for another three-year period. To do so, the results of a test of all herds in which infection was reported at the time of the previous certifying test or since, together with the results of a test of 20 percent of other representative herds, must reflect a rate of infection which does not exceed one percent of the cattle and five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same 20 percent previously tested for this same purpose.

B. Area certified under the provisions of Part IV, Section I “A” or I “B” may be continued as certified with the application of semi-annual milk ring tests, follow-up blood tests of milk reacting herds, and blood tests at three-year intervals on 20 percent of all herds not included in the milk test, if the incidence of infection does not exceed one percent of the cattle, and five percent of the herds.

C. (1) At the expiration of the three-year period, range and semi-range areas may be certified for another three-year period when at least 20 percent of the herds, including animals as outlined under Part IV, Section I, C “1” have been retested and the animal infection rate does not exceed one percent in not more than five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same group previously tested for this same purpose.

(2) Modified Certified Brucellosis-Free range or semi-range areas may be maintained in a certified status for additional periods of three years, provided:

(a) That at least eighty percent of the heifer calves retained in the area annually are officially vaccinated; providing that vaccination is not mandatory in strictly range areas where winter feeding is not practiced.

(b) That during each year at least five percent of the breeding cows in the area, as determined by the statistics of Agricultural Market Service, or a total of fifteen percent during a three-year period, are subjected to the agglutination test for Brucellosis; the blood samples to be taken from cull and slaughter cows at ranches, sale yards, or slaughtering establishments.

(c) That herds of origin of cattle reacting at a Titer of complete at 1-100 are subjected to an official blood test, and are handled according to the provisions of Part IV, Section I C(3).

(d) That herds found infected during the preceding certification period are blood tested at least one year following release from quarantine.

(e) That dairy herds in the area are screened semi-annually by the milk ring test, with blood test of herds reacting to the milk ring test.

(f) That the percentage of infection disclosed as a result of such tests as conducted under the provisions of the above four paragraphs does not exceed five percent of the herds, and one percent of the area cattle population over six months of age (excluding steers and spayed heifers); the number of reactors used in computing the percentage to be the number accumulated over the three-year period.

D. If testing as outlined under Part IV, Section II “A”, II “B”, II “C” (1) or (2)
reveals an animal infection rate of more than one percent, but not over two percent and a retest of the infected herds applied within 120 days discloses not more than one percent animal infection in not over five percent of the herds, the area may then be recertified.

E. Any area not qualifying for recertification under the provisions of Part IV, Section II shall be required to re-establish its certified status through testing procedures as outlined under Part IV, Section I.

SECTION III. Additions to Certified Areas

A. Cattle from officially certified brucellosis-free herds and cattle from negative herds in Modified Areas, when officially blood tested with negative results within one year of the date of shipment, may enter other Modified Certified Areas without being retested for brucellosis. All such cattle shall be individually identified and shall be accompanied by approved certificates of health indicating herd and animal status.

B. Cattle from herds under Federal-State supervision for the control of brucellosis may enter a Modified Certified Area or an area in the process of such certification when all animals in the herd were negative to the official blood agglutination test for brucellosis within 90 days of the date of entry. Individual animals to be moved must be negative to an official retest at least 30 days from the date of the previous herd test and within 30 days of entry or be official vaccinates under 30 months of age.

C. Cattle under 30 months of age officially vaccinated and coming from (a) negative herds in Modified Certified Areas, (b) individually certified brucellosis-free herds, or (c) herds under Federal-State supervision which have passed a test as under paragraph “B” may enter a Modified Certified Area or an area in the process of certification without further test when individually identified by mark, brand, tattoo, or other acceptable identification, and approved by the proper sanitary official of the State of origin.

D. Breeding cattle not over 30 months of age, officially vaccinated which do not qualify under paragraph “C” may enter a Modified Certified Area providing they do not show blood agglutination reactions higher than incomplete in dilution of 1–200 and the animals are maintained in quarantine until they have passed a negative blood test.

E. All other male or female cattle over six months of age, 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 30 days prior to the date of entry. They shall be maintained in quarantine separate from other cattle and retested in not less than 30 nor more than 60 days after date of entry. If retested and classed negative, they shall be released from quarantine.
REPORT OF THE DELEGATE FROM THE UNITED STATES
LIVESTOCK SANITARY ASSOCIATION TO THE
NATIONAL BRUCELLOSIS COMMITTEE

ROBINSON W. SMITH, D.V.M.

Concord, New Hampshire

The annual meeting of the National Brucellosis Committee took place at the
LaSalle Hotel, Chicago, Illinois, on Thursday, February 16.

The meeting was called to order by Chairman Herman C. Aaberg at 9:30 A.M.

The following members and guests were in attendance:

Herman C. Aaberg
Bill Allan
Thomas F. Arnold
R. D. Barner
Charles E. Bell, Jr.
R. W. Boone
D. C. Boughton
H. S. Bryan
Don Button
Acord Cantwell
J. V. Cavanaugh
C. F. Clark
J. W. Cunkelman
Lee Davisson
Aubrey D. Gates
W. A. Hagan
Radford Hall
E. J. Haslerud
Robert D. Havener
K. K. Heideman
R. A. Hendershott
John B. Herrick
H. E. Kingman, Jr.
Frank Knutsen
A. K. Kuttler
Walter H. Lloyd
Richard M. Luther
C. A. Manthei
C. K. Mingle
Joe G. Montague
Doug Mossberg
J. R. McMahan
S. H. McNutt
C. F. Neumann
H. S. Nicol
Roy B. Ormond
Greg. Pietrzszek

American Farm Bureau Federation
Radio Station KSTT
American National Cattleman's Association
Michigan State University
Federal Extension Service, U.S.D.A.
Agricultural Research Service, U.S.D.A.
DuPont Company
University of Illinois
National Livestock Producers Assoc.
Indiana Farm Bureau Fed.
Purebred Dairy Cattle Association
Adv. Com. to U.S.D.A.
Swift and Company
Michigan State Department of Agriculture
American Medical Association
U.S.D.A. Brucellosis Committee
American National Cattleman's Association
Extension Service
Ohio State University, Dept. of Animal Science
American Farm Bureau Federation
State Department of Agriculture, Trenton, N.J.
Iowa State College
American Veterinary Medical Association
Swift and Company
Animal Disease Eradication, U.S.D.A.
Livestock Conservation, Inc.
Animal Husbandry Dept., S. Dakota State College
Disease and Parasite Research, U.S.D.A.
Agricultural Research Service, U.S.D.A.
Texas & Southwestern Cattle Raisers Assn.
Livestock Conservation, Inc.,
Chas. Pfizer & Company
University of Wisconsin
National Live Stock and Meat Board
Iowa Farm Bureau Federation
Oscar Mayer & Company
National Provisioner
Chairman Aaberg introduced Mr. Walter Lloyd who has been acting as Secretary since Dr. Pickard's resignation from Livestock Conservation, Inc.

Mr. Lloyd stated that since Dr. Pickard's resignation from Livestock Conservation, Inc., he had tried to "keep the wheels turning" and he expressed his appreciation to Mr. Aaberg for his fine cooperation and help. Livestock Conservation, Inc., has been active nationally in a publicity way and has had several feature articles published, regarding brucellosis by the livestock press with the help of Dr. Van Houweling. Copies of the literature which were issued at the last meeting have been distributed by the thousands. Livestock Conservation, Inc. was asked to act as a liaison between the Agricultural Research Service and the various livestock groups around the country, on livestock diseases. We have participated as best we could in the questionnaire survey sent out by the Hagan committee. We could not take a positive stand but did circulate the questionnaire and filed a report. We have had two conferences on interstate regulations, largely as a means of acquainting member organizations of the National Brucellosis Committee with the proposals so that their organizations could speak for themselves on this matter.

Chairman Aaberg said that a number of committee members suggested that a recognition program be developed for those who have given outstanding service in eradication brucellosis and that since Dr. A. K. Kuttler was changing work, Mr. W. D. Knox was asked to prepare a scroll for him.

Motion was made, seconded and carried that a scroll be presented to Dr. Kuttler for his outstanding work in the field of brucellosis eradication.

Chairman Aaberg then gave in part the following report:

We have enjoyed another year of significant progress in the goal to eradicate brucellosis in the United States not later than 1960. Although much of this progress is due to the additional funds made available to finance the program, due credit must be given to the splendid support of all segments of the livestock and dairy
industry and to all other groups and agencies having an interest in the program. Much credit is due also to the leadership and work of many state and local committees, through the development of comprehensive educational programs leading to better understanding on the part of farmers and ranchers.

It is gratifying to note that all states have now qualified to participate in the accelerated program to eradicate brucellosis and have developed cooperative agreements with the Federal Government.

A significant development in recent months is the recognition on the part of several important farm and livestock groups that brucellosis can and should be eradicated not later than 1960 and that necessary State and Federal funds should be provided. I should like to quote resolutions on this question adopted by three important groups, as follows:

**American National Cattlemen's Association:**

Whereas we recognize that the public health of our nation necessitates eradicating brucellosis from beef cattle as well as dairy cattle; therefore, be it resolved that we suggest 1960 as a goal at which time brucellosis in the United States would be eradicated and that adequate state and federal funds be provided for both research and eradication; also we request prompt recognition by each state and the U.S.D.A. of areas completely free of brucellosis in addition to the now recognized, modified, certified brucellosis free areas; also, we urge our members to carry on an accelerated calfhood vaccination program in range areas in order to make a complete eradication less costly when ultimately achieved!

**The National Grange:**

The National Grange supports an accelerated brucellosis program, including increased indemnity payments for losses to producers. We recommend that states and producers cooperate to the fullest extent in carrying such a program.

**The American Farm Bureau Federation:**

We urge that the program to eradicate brucellosis be accelerated, that it be completed by 1960, and that adequate state and federal funds be provided immediately where necessary, to accomplish this.

The appointment of a special committee by the Secretary of Agriculture to study the program to eradicate brucellosis, under the capable leadership of Dean Hagan and other members of the committee, including W. D. Knox, Tom Arnold, and Dr. Van Houweling, should prove helpful in bringing about further improvements in the program.

At a luncheon this noon, we plan to honor a man who has made a significant contribution to the program of eradicating brucellosis. The idea of giving recognition to outstanding leaders in this field is a good one. May I suggest that plans be made to give recognition at subsequent annual meetings of the National Brucellosis Committee to other outstanding individuals. I believe it would be a good idea also to give special recognition to the leadership in the states as brucellosis is eradicated in such states. I am hopeful if such a recognition program is adopted that all states will have been included by 1960. We should then plan a real celebration at that time.

**REPORT OF THE SUBCOMMITTEE ON INFORMATION AND EDUCATION**

**NATIONAL BRUCELLOSIS COMMITTEE, CHICAGO, FEB., 1956**

*Presented by George E. Parsons, Chm.*

The Subcommittee appreciates that during the past year great progress has been made in brucellosis education work, and expresses its thanks to all those who helped make this possible.
The Subcommittee makes the following recommendations to the National Brucellosis Committee:

1. We recommend that materials be collected on the state brucellosis programs plus step-by-step information on how these brucellosis programs were implemented and operated within the respective states. This material is to be assembled and organized by the joint efforts of Charles E. Bell, Miss Virginia Tatum and Dr. C. K. Mingle, prior to a meeting with a state Extension dairyman, state Extension veterinarian, state Extension animal husbandman and a member of the farm press, at which time the material will be put into a final form for distribution to all the states. The appointment of the three-state Extension representatives and the farm press shall be made by the chairman of the National Brucellosis Committee in cooperation with the chairman of the Subcommittee on Information and Education.

2. The committee in recognizing the effectiveness of the film "Triple Threat" asks that the Animal Disease Eradication Branch of the ARS develop a new film on brucellosis eradication. This film is to depict the actual implementation of the program and operation of that program including the use of new techniques and procedures.

3. The committee recommends that the chairman of the National Brucellosis Committee contact the National Association of Farm Paper Editors and the National Association of Farm Radio Editors asking permission to present brucellosis information at their annual meetings, and to explore the possibilities of these national Associations becoming members of the National Brucellosis Committee.

4. The committee recommends that the chairman of the National Brucellosis Committee suggest to the state brucellosis committees the establishment of an Information and Educational Subcommittee within their state.

5. The committee recommends the promotion of the slogan setting forth the 1960 goal of a brucellosis free state.

6. The committee recommends that the chairman of the National Brucellosis Committee take all steps necessary to fully acquaint all vocational agricultural instructors with brucellosis eradication Programs. The contact to be made through the state brucellosis committees.

7. The committee recommends that the National Brucellosis Committee consider an award program for recognizing those individuals or groups making an outstanding contribution to the eradication of brucellosis.

8. The committee recommends a stepped-up educational program in brucellosis eradication in swine.

9. The committee recommends a release to news media evaluating various state's progress in control as compared to general level.

THE CONTROL OF BACILLARY HEMOGLOBINURIA

L. D. S. SMITH, PH.D.

Bozeman, Montana

Most of our knowledge of bacillary hemoglobinuria in cattle we owe to Vawter and Records, who studied this disease for many years in Nevada. These workers demonstrated the cause to be *Clostridium hemolyticum* and developed a specific serum for its treatment and a vaccine for its prevention. They also demonstrated that the disease, as it occurs in the field, is most difficult to reproduce experimentally. Administration of living cultures, either intravenously or by mouth, resulted in no visible effects. The only occasion on which typical clinical symptoms including hemoglobinuria were obtained, occurred after the intravenous injection of culture followed by an intrahepatic inoculation of calcium chloride solution four days later. Apparently, some damage to liver tissue is necessary before *Clostridium hemolyticum* can start to grow in this organ.

In Montana, we have had this disease since 1939. It has been restricted to high mountain valleys and from the original focus of infection has spread to four other areas involving approximately 1500 square miles or about one percent of the area of the state. This has presented an opportunity for observations on the spread of a clostridial disease. It appears that bacillary hemoglobinuria is generally spread by direct animal contact, contaminated water, and by carrier animals. The spread by direct animal contact and by contaminated water probably serves to infect contiguous areas; the infection of non-contiguous areas is apparently by the movement of carrier animals. The field evidence for the importance of carrier animals was supported by the finding that apparently healthy cattle could carry *Clostridium hemolyticum* in their livers. Such animals apparently had some immunity against this organism, for their sera always contained agglutinins for *Clostridium hemolyticum* and usually also contained antitoxin for the lethal toxin of this organism.

These findings indicated that serological tests might be valuable in controlling the spread of bacillary hemoglobinuria from one area to another, particularly since *Clostridium hemolyticum* has never been found in nature except in animals suffering from clinical or subclinical infections, and since none of the commonly occurring clostridia bear antigens in common with it.

The agglutination test is valueless for the detection of infection in vaccinated cattle since they respond to the injection of bacterin with a marked agglutinin response, but it was considered that it might be of value in detecting unvaccinated carriers. Since the boundaries of the areas in which bacillary hemoglobinuria occurred were sharply defined, it was possible to test the reliability of the agglutination test in unvaccinated animals in infected and non-infected areas. Sera from 306 unvaccinated animals in 14 herds in infected areas were tested and agglutinins for *Clostridium hemolyticum* were found in 26.1 percent of them. Sera from 112 cattle in six herds in non-infected areas were tested and agglutinins were found in 19.6 percent of them.
These results indicated either that *Clostridium hemolyticum* was spread far beyond the area where bacillary hemoglobinuria was known to exist, or that some other organism bearing antigens in common with *Clostridium hemolyticum* was widely distributed and occasionally caused inapparent infections. The solution of this problem was greatly aided by the discovery of a pasture that was apparently contaminated with such organisms and which was more than one hundred miles from the nearest area where clinical cases occurred. The causes of death of all cattle that had perished on this ranch for many years was known, and it could be stated to be free of bacillary hemoglobinuria as definitely as that statement could be made of any farm.

Twenty-two year-old Hereford cattle obtained from a region in which bacillary hemoglobinuria had never been found were bled and moved to this pasture in the spring of 1954. No agglutinins could be demonstrated in these sera. Six weeks later, however, agglutinins were demonstrated in the sera of 18 of the 22 animals. In 1955, 25 cattle were placed on the pasture after samples of sera had been obtained. One serum of the 25 contained agglutinins for *Clostridium hemolyticum*. Five months later the sera of 23 of the 25 animals were found to contain agglutinins. Apparently, this pasture was seeded with organisms bearing antigens in common with *Clostridium hemolyticum* and the cattle were responding to subclinical infections by this organism.

Two of the animals that had grazed on the pasture in 1954 and two that had grazed on it in 1955 were slaughtered and cultures were made from the livers. From each of these animals was isolated a clostridium that bore antigens in common with *Clostridium hemolyticum*. They resembled it in other respects, also. Morphologically, they could not be distinguished, and culturally these variants differed from typical strains of *Clostridium hemolyticum* only in their ability to ferment maltose. Serologically, agglutinin-adsorption tests demonstrated that while these variants were not identical with *Clostridium hemolyticum*, they did share a number of antigens with it. So far as toxin production was concerned, the variants produced a lecithin-digesting toxin serologically similar to that produced by *Clostridium hemolyticum*. However, the variant strains were poor producers of toxin, forming only five to 10 percent as much as did strains of *Clostridium hemolyticum* in the same medium. Likewise, they were of markedly lower virulence, for only occasionally could fatal infections in laboratory animals be elicited by intramuscular injection.

We concluded, from the results of these investigations, that these variants were relatively non-virulent strains of *Clostridium hemolyticum*, that they were widespread outside of the areas infected with bacillary hemoglobinuria, and that the agglutination test could not be used, even in unvaccinated cattle, for the detection of carriers of virulent strains of *Clostridium hemolyticum*.

The results of preliminary experiments indicated that the sera of carrier animals contained antitoxin, and that vaccination of cattle with commercial bacterin did not stimulate the production of antitoxin. Consequently, it was considered possible that an antitoxin test might be useful in the detection of carrier animals even though they had been vaccinated. Further investigation, however, ruled out this possibility, also, for one heifer was found to be carrying
a virulent strain of *Clostridium hemolyticum* although no antitoxin could be demonstrated in the serum. Furthermore, when commercial bacterin produced by eight different manufacturers was tested in cattle, it was found that the bacterin of two manufacturers caused the production of antitoxin in 10 to 20 percent of the cattle. It was concluded, then, that neither agglutination nor antitoxin tests could be used to detect carrier animals, and that no control measures could be applied so far as this aspect of the problem was concerned.

The vaccine developed by Records and Vawter has been used for many years for protection against bacillary hemoglobinuria. Although vaccination at yearly intervals is sufficient in some parts of the United States, vaccination every six months proved to be necessary in the mountain valleys of Montana. This may be due to continuous grazing of cattle on contaminated pastures. Beyond six months following vaccination, the immunity does not seem to disappear completely, but probably drops to a level below that necessary for complete protection. In such cases, hemoglobinuria is often absent and death does not occur until a week or more after the first symptoms. The characteristic liver infarct is always present, however, and *C. hemolyticum* is readily isolated from the liver.

Continual re-vaccination apparently serves to reduce the infectivity of infected areas. In the first area in Montana to become infected, vaccination was routinely practiced on most of the ranches for six to eight years. Although several of these ranches had once been heavily infected, to judge from the incidence of the disease before vaccination was started, cessation of vaccination did not lead to immediate outbreaks. Several years after vaccination had been discontinued a few cases were again encountered, indicating that the organism was still present. There is no reason to believe that *Clostridium hemolyticum* can be eradicated from an area by a program of continual re-vaccination, however, although the rate of infection may be markedly reduced and clinical cases may be almost entirely eliminated.

Vaccination has not proved to be uniformly effective in all of the areas in which bacillary hemoglobinuria has made its appearance. In one mountain valley in which bacillary hemoglobinuria was introduced in 1946, six month's protection is not always afforded by vaccination. Numerous cattle in this valley have succumbed to bacillary hemoglobinuria within six months of vaccination. The shortest time to elapse between vaccination and death was six weeks, and many cattle have succumbed three to four months after vaccination. This short-lived immunity is not associated with the bacterin of any one manufacturer, for a number of brands have proved unreliable in this area in spite of the fact that they are dependable in the other infected areas of the state. We do not know of any brand of *Clostridium hemolyticum* bacterin that may be used with certainty of six month's protection in this area.

Strains of *Clostridium hemolyticum* isolated from cases in recently vaccinated animals do not differ from classical strains in the serological specificity of the toxin that they produce, although all of them produce large amounts of toxin. The somatic antigens of these strains have not as yet been compared with those of classical strains, although it is known that there is considerable cross-agglutination.
There is one further possible method of control of bacillary hemoglobinuria that has not been explored. From the work of Records and Vawter, it appears that some damage must be done to the liver before *Clostridium hemolyticum* can start to grow in this organ. In Montana, it appears that the most likely cause of this damage is the liver fluke, *Fasciola hepatica*. All the epizootiological evidence we have collected points toward an association of bacillary hemoglobinuria with liver fluke infestation, although it should be pointed out that Records and Vawter were unable to demonstrate any such association. If liver fluke infestation is necessary for the development of *Clostridium hemolyticum* in the liver, it should be possible to control bacillary hemoglobinuria by eradication of the liver flukes. The methods now available for fluke eradication are, unfortunately, not applicable to the conditions in mountain valleys and further work will have to be done before this hypothesis can be tested.

In summary, there is no method now available that can be used for the detection of either vaccinated or unvaccinated cattle that are carrying *Clostridium hemolyticum*; the disease may be very largely prevented by routine vaccination, and this will not only protect the individual animals but will probably also reduce the contamination of the premises; and finally, vaccination with the present commercial bacterins does not give equally good results in all areas.
FURTHER STUDIES WITH LEPTOSPIRA POMONA BACTERIN

T. Burnstein, D.V.M., Ph.D., S. F. Scheidy, V.M.D.,

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania

There is little doubt that leptospirosis, as a disease of considerable economic
importance in our domestic animal population, should be controlled. At the
present time, although the therapeutic approach appears promising, prophylaxis
by vaccination seems to be the only satisfactory way of adequately controlling
the disease.

The literature contains several reports which show the effectiveness of bac-
terins in protecting cattle against leptospirosis as caused by Leptospira po-
mona. (1-3) Brown et al. (2) have shown that cattle are immune to challenge
at least six months after vaccination. It would appear important to determine
whether the duration of immunity is greater than six months.

It is generally agreed that L. pomona is also the primary cause of porcine
leptospirosis. Thus many veterinarians use the bacterin, which was originally
developed for the bovine disease, to vaccinate swine. There has been, however,
essentially no published information on the use of the bacterin in pigs.

The experiments to be described in this report are divided into three sections
which consider: (a) the duration of immunity in cattle; (b) vaccination of young
pigs; (c) the effects of challenge on the immunity of vaccinated guinea pigs.

MATERIALS AND METHODS

Bacterin

The bacterin used in these studies came from several production lots.* Details
on its preparation have been described previously. (1) Cultures used for the
preparation of the bacterin contained from 800 million to one billion leptospires
per ml.

Challenge Strains of L. pomona

Strain M.L.S., carried in guinea pigs, was used to challenge cattle.
Strain 14927† carried in guinea pigs was used to challenge both vaccinated
swine and guinea pigs.
Strain 14927 carried in Stewart's medium was used for guinea pigs also. This
strain in culture retains its virulence for swine and guinea pigs.
Challenge inoculum made up with strains which were carried in guinea pigs
was prepared by pooling defibrinated blood from at least three guinea pigs at
the height of leptospiral infection. Cattle received 2 ml. each injected subcu-
taneously, whereas each pig was inoculated with 1.5 ml. by the same route.

*‘Antilepto' Leptospira pomona bacterin prepared by the Biological Production
Laboratories, Merck Sharp & Dohme Division, West Point, Pennsylvania.
† Isolated from swine by Dr. H. Bryan, University of Illinois.
Isolation of Leptospires

For leptospiral isolation from challenged animals, blood or urine samples, or kidney suspensions were each injected into groups of three guinea pigs. Temperatures were taken daily. If a febrile response occurred in one or two of the group, these animals were sacrificed and observed for typical lesions and the presence of leptospires in the peritoneal fluids. If the latter were negative, the remainder of the group, and all animals not showing fever, were challenged with blood from a previously infected guinea pig passage. If challenged guinea pigs were refractory, the original sample was considered to contain leptospires.

Urine samples were examined at frequent intervals by darkfield microscopy as soon as possible after collection, usually within 10 minutes.

Serology

The microscopic agglutination-lysis test was employed to detect leptospiral antibodies. The antigen used was a young culture of *L. pomona*, strain T-262, grown in modified Stewart's medium with 10 percent rabbit serum. Equal portions of inactivated serum, appropriately diluted, and antigen were mixed and incubated for three hours at 30°C. Tests were read at 150× magnification using a darkfield microscope.

Titers are presented as the reciprocal of the final dilution of serum which agglutinated or lysed the antigen. Tests on all serum samples from one experiment were conducted at the same time with the same batch of antigen.

PART I: STUDIES ON THE DURATION OF IMMUNITY IN CATTLE

Considering the lack of knowledge regarding the epizootiology of leptospirosis, long-term immunity studies must be evaluated cautiously. Animals after vaccination and before challenge may conceivably be exposed to leptospires in a number of ways, e.g. through drinking water, feed, rodents, other wild animals, caretakers, etc. The ideal experiment would be to place the animals in isolation units after vaccination. However, this is not practical.

Recognizing the possible complicating factors in this type of experiment, our approach was to vaccinate animals with a single dose of bacterin; bleed them at selected intervals after vaccination; and then challenge them with a virulent strain of *L. pomona*. Cattle selected for these experiments had no detectable leptospiral antibodies prior to vaccination. After vaccination, animals were kept in isolation from other animals on farms which had no previous history of leptospirosis. At the time of challenge, animals were brought into our research barns for completion of the studies. Appropriate controls were provided to insure that the challenge inoculum was virulent. Criteria for infection in vaccinated and control animals were clinical signs, isolation of leptospires when possible, and serological response.

Three separate experiments were conducted. In the first, five dairy-type animals vaccinated with a single 5 ml. dose were challenged six months after vaccination. In the second trial, five dairy-type cattle were challenged 12 months post-vaccination. In the third test, 10 beef-type animals were challenged 14 months
after vaccination. For serology, cattle were bled at selected intervals until they were challenged and then weekly until experiments were terminated. In Experiments I and II body temperatures of challenged animals were taken daily. At the first sign of fever, animals were bled for leptospiral isolation. Several urine samples were also collected for isolation of leptospires. Observations for the characteristic nephritis of leptospirosis were made only in animals in Experiment II. In Experiment III, challenged animals were kept on pasture, and it was difficult to observe them for clinical effects other than frank illness. Thus serological response was of primary importance in these animals.

Results

The results of these experiments are presented in Tables 1 and 2. Experiments will be discussed individually.

Experiment I: Vaccinated animals challenged at six months did not become clinically ill with leptospirosis. The three controls, however, showed a rise in body temperature varying from 104.4 to 106°F. The controls had only the mild form of the disease, since they recovered after two or three days of illness. Isolations were made from the blood of all of the controls but from none of the vaccinated group. As can be seen in Table 2, the antibody levels of the vaccinated cattle were little affected by the challenge inoculum. On the other hand, titers of the controls rose to levels above 1:10,000. It was interesting to note that two vaccinated animals with no detectable titers were resistant to challenge.

Experiment II: The results were similar to those of the six-month test. Since the animals were sacrificed at the completion of the experiment, attempts were made to isolate leptospires from the kidneys as well as the blood and urine. No isolations were made from any of the five vaccinated animals. However, leptospires were isolated from the blood, urine and kidneys of two of the three control animals. The latter also had lesions of interstitial nephritis. Serologically, vaccinated animals did not respond to the challenge, whereas the titers of all the controls again rose to above 1:10,000.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Interval before Chall.</th>
<th>No. of Cattle</th>
<th>Fever or other Signs</th>
<th>Isolation of Leptospires</th>
<th>No. Immune Based on Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6 mo.</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>12 mo.</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>14 mo.</td>
<td>10</td>
<td>N.T.4</td>
<td>N.T.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7*</td>
<td>N.T.</td>
<td>N.T.</td>
<td>0</td>
</tr>
</tbody>
</table>

1 From blood and/or urine.
2 Low serological response compared to that of controls.
3 Controls.
4 Not tested.
TABLE 2

Serological Response of Vaccinated Cattle to Challenge

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Interval before Chall.</th>
<th>No. of Cattle</th>
<th>Titer at Chall.</th>
<th>Highest Titer Post-Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6 mo.</td>
<td>5</td>
<td>2-0</td>
<td>4-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-10</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td>1-50</td>
<td>1-50</td>
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<td></td>
<td></td>
<td></td>
<td>3-0</td>
<td>3-&gt;10,000</td>
</tr>
<tr>
<td>II</td>
<td>12 mo.</td>
<td>5</td>
<td>2-0</td>
<td>3-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-10</td>
<td>2-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-0</td>
<td>3-&gt;10,000</td>
</tr>
<tr>
<td>III</td>
<td>14 mo.</td>
<td>10</td>
<td>3-0</td>
<td>1-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-10</td>
<td>5-50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-50</td>
<td>4-250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7-0</td>
<td>5-5,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-10,000</td>
</tr>
</tbody>
</table>

1 Controls.
2 No. of animals with their titers. Titers are given as reciprocal of dilution.

Experiment III: The criterion for protection in this test was serological response. As can be seen in Table 2, the vaccinated animals responded only weakly to the challenge inoculum as compared to the controls. The titers of the controls were not as high as in the other experiments. Nevertheless, the difference is significant. Again, it should be noted that vaccinated animals with no detectable antibodies resisted infection.

PART II: VACCINATION OF YOUNG PIGS AGAINST LEPTOSPIROSIS

Two experiments were conducted to determine the following: (a) the magnitude of serological response after a single 5 ml. dose of bacterin; (b) the ability of vaccinated pigs to resist challenge.

Cross-bred Yorkshire-Hampshire pigs, eight weeks of age were used. They were raised on our own farm and were known to be free from leptospirosis. They were fed a commercial ration, free of antibiotic supplements.

In Experiment I, the bacterin was administered to 19 pigs subcutaneously. In Experiment II, nine pigs were injected subcutaneously and nine intramuscularly. Animals were bled at weekly intervals for serological studies.

Fourteen of the pigs in Experiment II and five unvaccinated controls were challenged 48 days after vaccination. Animals were placed in isolation units with the controls separated from the vaccinated pigs. Body temperatures were recorded twice daily. Within 24 hours of the time that any of the controls showed a rise in temperature, all controls and the six vaccinated pigs with the highest temperatures were bled for leptospiral isolation. Three urine samples from each of the same 11 animals were also tested for leptospires by guinea pig inoculation. Urines from all pigs were examined frequently with a darkfield microscope.
Blood also was collected for serology. Five weeks post-challenge, all pigs were sacrificed and necropsied. Kidney suspensions from the six vaccinated animals that were tested for leptospiral isolation were inoculated into guinea pigs. Since leptospirosis had been demonstrated in the controls at the time of death, therefore it was considered unnecessary to test the kidneys for leptospires.

Results

In the challenge experiment, seven to eight days after inoculation, four of five controls responded with abnormal temperatures ranging from 104.5 to 105.8°F. The temperature response lasted from several hours to two days. Leptospires were isolated from the blood of four of the controls. Urine samples from the same four controls contained living leptospires on the 13th day post-challenge. The fifth control showed neither leptospiremia nor leptospirosis. The vaccinated pigs did not show temperatures above 103.6°F. The six vaccinated animals tested for leptospiremia at the time their temperatures were highest (103.2–103.6°F) were negative. None of the vaccinated animals was found to have leptospirosis.

At necropsy, the only changes noted were in the kidneys of the controls. The four controls from which leptospires had been isolated had very extensive interstitial nephritis. The surfaces of the kidney were covered with numerous whitened areas of various sizes. These are typical of the changes seen in the convalescent stage of the experimental infection.

The clinical and laboratory data are presented in Table 4.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Agglutination-Lysis Titers of Pigs Three Weeks after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
<td>No. of Pigs</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>19</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Signs of Leptospirosis in Vaccinated Pigs Challenged with Virulent Leptospires</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rise in Temp.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>0/14</td>
</tr>
<tr>
<td>Controls</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Numerator—No. positive.
Denominator—No. tested.
TABLE 5
Serological Response of Pigs Following Challenge

<table>
<thead>
<tr>
<th>Route of Vac.</th>
<th>No. of Pigs</th>
<th>Titer at Chall.</th>
<th>Maximum Titer Post-Chall.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcut.</td>
<td>7</td>
<td>4–10</td>
<td>1–10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2–50</td>
<td>2–200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–250</td>
<td>1–500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2–2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–8000</td>
</tr>
<tr>
<td>Intramus.</td>
<td>7</td>
<td>1–10</td>
<td>3–100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3–50</td>
<td>1–500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3–250</td>
<td>2–800</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–4000</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>5–0</td>
<td>4–64,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–32,000</td>
</tr>
</tbody>
</table>

Serological data are presented in Table 3 which shows the response to the vaccination of the 37 pigs. All pigs had detectable antibody levels, titers varying for the most part from 1:10 to 1:250. One pig had a weak reaction at 1:1250. The degree of response of these pigs is similar to that of cattle although the pigs received considerably more antigen, on a weight basis, than the cattle. Titers were detectable on the sixth day and seemed to reach a peak between the 14th and the 21st day. The response of pigs receiving the bacterin subcutaneously did not differ significantly from that of animals vaccinated intramuscularly. Thus these animals are included together in Table 3.

Antibody studies on the challenged pigs are presented in Table 5. It can be seen that as a whole the titers of the vaccinated pigs following challenge were lower than those of the controls. However, it will be noted that some of the responses of the vaccinated group were rather high and, in one pig, reached a titer of 1:8000. The titers of the controls were high, including the one pig that showed no other evidence of leptospirosis. This animal had a titer of 1:64,000.

**PART III: STUDIES ON THE EFFECTS OF CHALLENGE ON THE IMMUNITY OF GUINEA PIGS**

Leptospirosis in many instances is a subclinical infection. Thus in the evaluation of challenge type experiments to test vaccine potency, one must rely on serological response. Ideally, immune individuals subjected to challenge would be expected to show essentially no rise in titer, whereas unvaccinated controls should show the typically high titer which follows natural infection.

In the preceding portions of this report, it was noted that vaccinated animals, although protected against experimental leptospirosis, varied in their serological response to challenge antigens. Thus in certain animals, the challenge inoculum elicited a rise in titer; while in others, titers were not affected by the challenge. Several pigs although apparently immune clinically to experimental infection showed a substantial increase in titer. One must ask if the rise in titer was due to replication of the spirochete, or only due to the booster effect of antigen pres-
ent in the inoculum. Furthermore, if replication should occur in certain vaccinated pigs, would it be sufficient magnitude to produce the clinical effects of leptospirosis? These questions are not easily answered.

Experimentally, immune systems can be overwhelmed by excessive amounts of replicating antigen. That is, in certain infections, if enough antigen is present in the challenge and replication occurs, the mere presence of serum antibodies does not necessarily mean that the challenged individual would be immune. In the swine experiments it is estimated on a weight basis that challenged pigs received five times the amount of antigen given to challenged cattle. It is conceivable that this increased amount of antigen was sufficient to overwhelm the immunity of the vaccinated pigs that responded with high titers. It must be kept in mind that the challenge given to these pigs is far more severe than that obtained in the field by natural exposure.

In the studies to follow, an attempt was made to answer the above question by trying to overwhelm the immunity of vaccinated guinea pigs. Guinea pigs were selected because they can be immunized and unvaccinated animals can be consistently and uniformly infected.

**Experimental**

Forty-seven guinea pigs (g.p.) weighing 250–300 Gm. were vaccinated subcutaneously, each receiving 1 ml. of bacterin which had been diluted in Stewart’s medium. Dilutions inoculated were: 1:10 into seven g.p.; 1:20 into 10 g.p.; 1:30 into 10 g.p.; 1:40 into 10 g.p.; and 1:50 into 10 g.p. Animals were bled three weeks later and their sera were tested for antibodies. Table 6 summarizes their responses:

As would be expected, the data in Table 6 suggest that the degree of response is a function of the amount of antigen in the inoculum, since the animals receiving less bacterin had weaker responses.

In several experiments, 34 of the above animals and 11 controls were divided into groups and challenged with three different inocula. These were: (a) living culture killed with Thimerosal; (c) infectious g.p. blood, the number of organ-

<table>
<thead>
<tr>
<th>Dilution of Bacterin</th>
<th>No. Vac.</th>
<th>No. Neg.*</th>
<th>No. Showing Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:10</td>
</tr>
<tr>
<td>1:10</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1:20</td>
<td>10</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>1:30</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1:40</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1:50</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* Negative at 1:10.
TABLE 7

Response of Vaccinated Guinea Pigs to Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of G.P.</th>
<th>Pre-Chall. Titer</th>
<th>Type of Chall.</th>
<th>No. with Fever</th>
<th>Post-Chall. Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>3-0</td>
<td>Live Culture</td>
<td>0</td>
<td>8-1250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-10</td>
<td></td>
<td></td>
<td>7-5000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>6-0</td>
<td>Live Culture</td>
<td>6</td>
<td>4-1250</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-5000</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>5-10</td>
<td>Killed Culture</td>
<td>0</td>
<td>1-50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-50</td>
<td></td>
<td></td>
<td>3-250</td>
</tr>
<tr>
<td>IV</td>
<td>12</td>
<td>5-0</td>
<td>Inf. G.P. Blood</td>
<td>0</td>
<td>4-2500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7-50</td>
<td></td>
<td></td>
<td>8-10,000</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>5-0</td>
<td>Inf. G.P. Blood</td>
<td>5</td>
<td>4-1250</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-5000</td>
</tr>
</tbody>
</table>

isms unknown. The latter contained in a previous titration approximately 5,000 infectious doses per ml. for guinea pigs. Each g.p. received 1 ml. intraperitoneally which in the case of the blood represented ¾ of that given to the swine. Animals were bled two weeks after challenge.

Group I comprised 15 animals with titers at the time of challenge varying from 0 to 1:250. These were challenged with live culture.

Group II included five controls for Group I, and were given live culture. Group III consisted of seven animals which were challenged with killed culture. Group IV was made up of 12 animals challenged with infectious blood. Group V was the control for Group IV and comprised five g.p.

The experiments are summarized in Table 7.

Guinea pigs in Groups I and IV did not show a fever following challenge, and were therefore presumed to be immune to clinical leptospirosis. The controls (Groups II and V), however, were febrile, with body temperatures varying from 104.5 to 105.9°F. lasting from two–four days. It was interesting to note that three animals in Group I and five in Group IV had no detectable titers at challenge, yet were immune.

The serological data show that all vaccinated groups responded strongly to challenge and developed high titers. In fact, in Group IV the titers were higher than those of the controls. This is not easy to explain. The serological response of Groups I and II appear to be similar. The titers in Group III which was challenged with killed organisms, were apparently lower than those of Groups I and II.

The data are difficult to interpret. Several facts, however, stand out. The titers of all animals were similarly high. Thus if one were evaluating protection in these experiments solely on a serological basis, he would conclude that there was none.
The quantity of antigen received by these animals in the inoculum was undoubtedly large. This in itself was responsible for part of the antibody response, as shown by the titers of Group III. Titers of the other groups suggest that a certain amount of replication occurred, since the values of the controls and vaccinated animals were similar.

**DISCUSSION**

Cattle were shown to be immune to leptospirosis as long as 14 months after vaccination with a single dose of bacterin. Protection was primarily based on the fact that challenge had essentially no effect on the animal serologically when compared to the controls. It would seem reasonable to conclude that the immunity was produced by the bacterin. As mentioned earlier, it is possible that vaccinated animals could have been exposed to extraneous leptospires during the prechallenge period, and that these organisms might have acted to stimulate and maintain immunity. We feel that this is unlikely, however, since precautions were taken to keep vaccinated animals in isolation. That one injection of bacterin could produce such prolonged immunity is unusual. It is difficult to speculate as to why this should occur. This is undoubtedly involved with the nature of the leptospiral protective antigen, which has not yet been characterized.

In the swine experiments, it was shown that a single dose of bacterin was capable of producing an antibody response in 37 young pigs. The degree of response was similar to that of cattle. Vaccinated swine challenged with a virulent strain of *L. pomona* which originally came from pigs showed no clinical signs of leptospirosis. The controls except for one animal showed a rise in temperature, leptospiremia, leptospiruria, and interstitial nephritis. Serologically the titers of most vaccinated pigs were little affected by the challenge. There was a slight rise in these pigs which was attributable to a booster effect. In several of the animals, however, there was a substantial rise in titers, although none was as high as that of the controls. It was felt that this rise was due to a certain amount of leptosprial replication. If this was so, it could be explained by the fact that there was a relatively large amount of antigen contained in the inoculum, and the antibodies present in the pig were not capable of coping with this replicating antigen. On a practical basis this probably would not occur in the field since the number of leptospires acquired by a pig naturally exposed would be far fewer than that contained in our challenge inoculum, unless the pig were to drink or inhale large quantities of infectious urine.

This led to the guinea pig experiments which were designed to determine whether the immunity of vaccinated guinea pigs could be overwhelmed by using heavily infected challenge inocula.

It was shown that vaccinated guinea pigs resisted the challenge since they did not become febrile. Serologically, however, titers of vaccinated animals were as high or even higher than the controls which would suggest that replication occurred in the vaccinated animals. This is further borne out by the fact that one group of vaccinated guinea pigs challenged with a killed antigen showed a weaker response than did the groups challenged with living antigen. Thus it is felt that the immune response serologically was overwhelmed. This points out
the danger of using too much antigen for challenge in potency-type experiments. Obviously one could not rely on or interpret serological data and especially in the case of leptospirosis which is essentially a subclinical disease.

In all these experiments, one interesting fact stands out. Many of the vaccinated animals had no detectable titers, and yet were immune to challenge. Thus immunity is not necessarily reflected by the presence or absence of demonstrable antibodies. This obviously has a number of practical implications.

**SUMMARY**

Cattle were immune to challenge as long as 12 to 14 months after vaccination with a single dose of bacterin.

Young pigs responded to vaccination with a single dose of bacterin by developing relatively low agglutination-lysis titers. A challenge experiment showed that these animals were immune to experimental leptospirosis.

Guinea pig studies were conducted to test the reliability of serological data in evaluating immunity of vaccinated animals. It was shown that if large numbers of replicating leptospires were used for challenge, titers of vaccinated animals were as high or higher than those of the controls, even though the vaccinated animals did not develop clinical leptospirosis.

The authors express appreciation to Dr. A. A. Creamer and Dr. R. S. Buchanan for their cooperation in making observations of some of the animals used in these studies.

**REFERENCES**


IMMUNOLOGICAL STUDIES ON INFECTIOUS BOVINE RHINOTRACHEITIS

CHARLES J. YORK, D.V.M., PH.D., AND ANTON J. F. SCHWARZ, M.D.

Indianapolis, Indiana*

It was in 1950 that an apparently new transmissible infectious disease of cattle was reported from western feed lot areas which was characterized by a high fever, excessive nasal discharge, respiratory distress, anorexia and depression. A similar disease was observed occurring in almost epidemic proportions in the dairy areas of southern California in late 1953. An excellent description and summary of this disease situation in Colorado and California was presented at the 1955 meeting of the United States Livestock Sanitary Association by McKercher (1) and Jensen (2). McKercher and Chow (3) also presented work with various experimental animals and embryonated eggs, but indicated that all efforts to isolate the causative agent of this disease had failed. McKercher showed by cross protection tests in cattle employing nasal washing material obtained from naturally infected cattle in California and Colorado that the disease was caused by a transmissible agent which appeared to be the same from both areas. At this meeting, it was generally agreed to call this condition “infectious bovine rhinotracheitis” (I.B.R.).

Since the 1955 meeting, a virus that is the cause of infectious bovine rhinotracheitis has been isolated in tissue cultures of bovine embryo kidney cells at about the same time by Madin in Berkeley, California, and York in Indianapolis, Indiana. A joint preliminary announcement of this work has been published (4). Further details on the isolation and growth of this virus by tissue culture methods have been submitted for publication elsewhere (5).

Immunological studies to demonstrate that this virus is the causative agent of I.B.R., and preliminary studies on how this virus has been modified in virulence to produce a practical vaccine are presented in this paper.

GROWTH OF THE VIRUS IN TISSUE CULTURE

Although details of the tissue culture work are to be found elsewhere, a brief review of this work is felt desirable. Tissue culture tubes consisting of trypsinized cells of bovine embryo kidney epithelium were routinely used. Initial isolation of the virus was made by inoculating into these tubes small quantities of nasal washings or suspensions of turbinate tissue obtained from cattle in the acute phase of I.B.R. disease. After 48 to 72 hours, the cells became obviously affected and then completely destroyed. The virus producing this cytopathogenic effect multiplied readily in these cultures, was easily transferred from culture to culture, and thus provided a means of studying its characteristics. The type of effect this virus has on tissue culture cells can be seen by a comparison of normal tissue culture of bovine embryo kidney cells (Figure 1) with a similar culture three days after inoculation (Figure 2).

* Research Department, Virus Research Laboratory, Pitman-Moore Company, Division of Allied Laboratories, Inc.
After the initial successful isolation of a virus from infectious material, a series of isolation attempts were made with various tissues and specimens from several animals naturally infected with I.B.R. A summary of this work can be seen in Table I.
As may be noted in Table I, this infectious agent was isolated from nasal washings, turbinate, larynx and tracheal tissue, but not from lungs, blood, spleen, or liver. While it may be possible that this virus appears for transient periods in other parts of the body, it is apparent that it has a primary predilection for the upper respiratory tract.

**IDENTIFICATION OF THE TISSUE CULTURE VIRUS**

The first step in showing that tissue culture virus is the etiological agent of the disease was obtained by inoculating a series of 20 cattle intranasally with infected tissue culture fluid. Uniformly after three to four days, a febrile response occurred ranging from 104 to 106°F., and generally lasting three to five days. Other signs of illness such as increased respirations, excessive nasal discharge, anorexia, depression, and patches of serofibrinous exudate clinging to the interior nasal wall, as well as hyperemia of the nasal mucosa could be observed. However, not all of these signs were present in all the animals. The picture of the disease seen in the experimental cattle resembled closely the illness found in naturally infected animals.

Further evidence that the tissue culture virus is the primary etiological agent was obtained from cross protection tests in cattle. In these studies, cattle immunized with one or another of three tissue culture viruses were challenged intranasally with known virulent virus capable of producing disease in susceptible control animals. This challenge virus consisted of either homologous tissue culture virus or other tissue culture isolates. Further challenges were made with known infectious nasal washings taken from naturally infected cattle. The results obtained may be seen in Table II.

As Table II shows, cattle immunized with Colorado 1 tissue culture virus did not respond to a challenge with the same virus, or with other tissue culture isolates such as Colorado 5 or Blythe. What is more important, other animals immunized with Colorado 1 tissue culture virus did not respond to a challenge with nasal washing material obtained from naturally infected cattle (Colorado 5), indicating that the infective material in the nasal washings was the same as the virus in the tissue culture fluid.

In order to determine whether the various strains of virus isolated from different parts of the country were the same immunological type, as well as to obtain addi-
TABLE II
Cross-Immunity Tests in Cattle with Several Strains of Infectious Bovine Rhinotracheitis Virus

<table>
<thead>
<tr>
<th>Immunizing Strain</th>
<th>Challenging Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colorado 1 T.C.</td>
</tr>
<tr>
<td>Colorado 4 N. W.*</td>
<td>1/1‡</td>
</tr>
<tr>
<td>Colorado 1 T. C.†</td>
<td>20/20</td>
</tr>
<tr>
<td>Blythe T. C.</td>
<td>1/1</td>
</tr>
</tbody>
</table>

* N. W. = Nasal washings from naturally infected cattle.
† T. C. = Tissue culture propagated virus.
‡ Numerator = Number of animals not reacting to challenge.
Denominator = Number of animals challenged.

Table II shows the cross-immunity tests conducted with different strains of infectious bovine rhinotracheitis virus. The immunizing strains were compared against various challenging strains to determine if cross-immunity occurred. The results are summarized in the table, showing the percentage of animals not reacting to the challenge.

Tissue culture proof that the tissue culture virus is the etiological agent, serum neutralization tests were conducted. Three virus strains isolated from widely separated areas of the country, and homologous as well as heterologous serum samples were employed. Samples from both naturally infected and experimentally inoculated cattle were used. Infected tissue culture fluid containing 100 to 1000 TCID₅₀ (50 percent tissue culture infectious doses) of virus was mixed with an equal quantity of undiluted serum to be tested, incubated for two hours at 37°C, and then 0.2 ml. of the mixture inoculated into each of several tissue culture tubes. This work is summarized in Table III.

TABLE III
Cross-Neutralization Tests with Three Strains of Infectious Bovine Rhinotracheitis Virus Using Homologous and Heterologous Immune Serums

<table>
<thead>
<tr>
<th>Strain of Virus Tested</th>
<th>Source of Neutralizing Serums</th>
<th>% of Serums Neutralizing the Viruses Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Natural Infection</td>
<td>Experimental Infection</td>
</tr>
<tr>
<td>Colorado Beef</td>
<td>1/1†</td>
<td>10/10</td>
</tr>
<tr>
<td>California Beef</td>
<td>1/1</td>
<td>7/7</td>
</tr>
<tr>
<td>California Dairy</td>
<td>1/1</td>
<td>7/7</td>
</tr>
</tbody>
</table>

* Normal serums = serum samples taken from experimental animals prior to inoculation with infectious bovine rhinotracheitis virus.
† Numerator = number of serum samples neutralizing virus; denominator = number of serum samples tested.
As may be noted in this table, each of the three viruses used—Colorado beef, California beef, and California dairy—were readily neutralized by homologous serum samples, whether naturally or experimentally produced. These strains were also neutralized equally well by sera of each of the other strains, indicating that they are all of the same antigenic type. A total of seven strains of virus have now been isolated and neutralized by at least one heterologous serum sample, suggesting that only one immunological type of virus is responsible for the disease as seen in various areas of the country. Serum samples from naturally occurring cases of I.B.R. neutralized the tissue culture isolates, furnishing additional proof that this virus is the causative agent of the disease.

DEVELOPMENT OF A VACCINE

The fact that animals, after recovery from the disease, successfully withstood a challenge with known virulent virus, and that all strains so far isolated appeared to be antigenically the same, showed it would be possible to immunize cattle against this infection if a vaccine could be developed.

In the course of passing one of the virus strains in tissue culture, various selective procedures were undertaken to determine whether the virulence of the virus could be altered. At various intervals during the process of carrying out these modification procedures, the virus was inoculated intramuscularly in a 1.0 ml. amount in cattle to test for virulence and antigenicity. All inoculated cattle were observed daily for signs of illness and febrile response. Serum samples were taken before inoculation, and again two or more weeks later. Usually three weeks after inoculation, the cattle were challenged intranasally with known virulent virus.

Twelve animals were inoculated with virus prepared during the early phases of the modification attempts. Eleven of these cattle developed a febrile response ranging from 103.5 to 106.5°F. which lasted for as long as four days. Increased respirations, anorexia and depression were also noted in a number of these animals. All these cattle, after recovery from the inoculation, developed antibodies and were resistant to challenge.

After the virus modification process had been completed, nine animals were inoculated intramuscularly and observed as described above. Only three of these showed any febrile response at all, and this lasted for one day only. Two of the responses were 103.6°F. and one was 105.4°F. No other signs of illness were observed. All these animals also developed antibodies and were resistant to challenge.

These facts demonstrate clearly that this attenuated virus can be used for a practical, safe and effective vaccination against I.B.R. The safety as well as potency of this live virus vaccine has been further established by large, controlled field studies, one of which is reported elsewhere in these proceedings (6). General field studies are in progress which will be published in detail at a later date.

Another important factor which has to be investigated in connection with the use of a live virus vaccine is the non-transmissibility of the virus. A series of susceptible control cattle was placed in contact with one or more intramuscularly inoculated cattle, in a small pen where they had to eat the same food and drink the same water. This contact lasted during the entire pre-challenge period, or approximately three to 10 weeks. None of the control animals developed antibodies,
and all were susceptible on challenge with virulent virus. This point has been further verified in the above-mentioned controlled field studies by employing large numbers of contact controls.

SUMMARY

The virus of infectious bovine rhinotracheitis has been isolated and propagated in bovine embryo kidney tissue culture. This virus could be used to reproduce the disease experimentally. Serum neutralization tests in tissue culture and cross protection tests in cattle offer further proof that the tissue culture virus is the etiological agent of the disease. All strains of virus so far isolated appear to be immunologically the same. By tissue culture procedures, one strain of virus has been modified so that, when inoculated intramuscularly in cattle, no signs of illness occurred but immunity developed. Furthermore, the virus did not spread from inoculated to susceptible contact cattle. These laboratory studies have led to the development of a safe and effective live virus vaccine for Infectious Bovine Rhinotracheitis.

REFERENCES

A CONTROLLED FIELD TRIAL OF A VACCINE FOR INFECTIOUS BOVINE RHINOTRACHEITIS*


Since 1955 when the etiological agent of infectious bovine rhinotracheitis (IBR) was isolated, there have been attempts to develop a vaccine against this disease. In the summer of 1956 a modified live virus vaccine was developed and successfully tested on small groups of calves kept under laboratory conditions.* It was decided to test the vaccine on a large group of feeder cattle kept under feedlot conditions.

Forty Hereford heifers weighing on the average, 650 lbs. were purchased from two sources. These animals were typical of those that are placed in feedlots for fattening. They were brought to the University of California School of Veterinary Medicine at Davis, where they were placed in a dry lot and fed alfalfa hay ad lib and three lbs. of rolled barley per day. The animals were branded and ear tagged for identification and a daily temperature was recorded on all animals during the entire course of the trial. Animals that exhibited a temperature of 104°F or more were stanchioned for a physical examination. Blood samples were taken from all animals when they arrived at Davis, on the day of vaccination and the day of challenge and were tested by serum neutralization test for the presence of infectious bovine rhinotracheitis antibody. On the initial test, nine animals from one of the two sources had antibodies and were discarded. Replacements were obtained from the other source and were found to be negative on the serum neutralization test so that all animals used in the trial had no detectable antibody against IBR.

Approximately three weeks after the cattle arrived and during which the initial neutralization tests were completed, the animals were divided into three groups. One group of 20 was vaccinated with two cc of IBR vaccine given intramuscularly. A second group of 11 was left unvaccinated but placed in the corral with the vaccinated animals. A third group was moved to another part of the ranch to be isolated and later serve as virus controls.

Twenty-three days after vaccination the vaccinated group of 20, the unvaccinated group of 11 that were left in contact with the vaccinated animals and four animals from the group held in isolation were challenged with a strain of IBR virus. This virus was isolated in California and had been passed on bovine embryo kidney tissue culture for three passages. The animals were inoculated by spraying 10 ml. of the virus containing tissue culture fluid into the nostrils of each animal with a household spray gun.

RESULTS

The average daily postvaccinal temperature and the extremes of temperature of the various groups are shown on graph I. From the second through the fourth

*Developed by the Virus Research Laboratory, Pitman Moore Company and reported at the United States Livestock Sanitary Association Meeting, November 1956.
day the average temperature of the vaccinates was one degree higher than the controls. For five days prior to vaccination and until nine days after vaccination, one or two animals each day experienced a temperature higher than 104.0°F. After vaccination all but two of these high temperatures occurred in the vaccinated group. Usually the temperature returned to normal the next day. Physical examination of these animals failed to reveal any symptoms other than an increase in pulse and respiration. While a transitory temperature rise may be expected after the use of a modified live virus vaccine, the appearance of fever in the controls, prior to vaccination, and at irregular intervals after vaccination, suggest that there was some other cause. Nasal swabs from eight of these animals were examined by tissue culture inoculation. A specimen from one animal yielded an agent not typical of the IBR virus and that could not be neutralized with IBR immune serum. All unvaccinated animals that experienced this idiopathic temperature rise later reacted to challenge. For these reasons it is certain that at least some of the temperature rises noted were due to unknown causes and not attributable to the vaccine. Few of these febrile animals would have been recognized had not daily temperatures been taken.

Twenty-three days after vaccination all vaccinated animals had antibodies against IBR, that could be demonstrated by serum neutralization test. The titers ranged from 1:3 to 1:50. None of the controls reacted to the serum neutralization test.
Graph II. Average daily temperature of all groups following challenge. All temperatures were between 100.0 and 109.0 and in noting the temperature range the first two digits of the number were omitted.

Graph number II shows that within 48 hours after challenge the controls began to show a temperature rise and within 96 hours all controls had developed a typical experimental IBR infection. There was some variation in severity and duration of infection but the graph of average temperatures shows the temperature curve typical of this disease as previously reported by McKercher, et al. (1) All controls exhibited nasal discharge, and a white fibrinous exudate firmly adherent to the nasal mucous membrane. There was stenosis of the nasal passage and dyspnea was easily induced when the animal resisted restraint. A few animals exhibited mouth breathing. Nasal swabs taken from three controls on the third day after challenge all yielded the virus when inoculated on tissue culture. Two of the controls died on the 10th day after challenge. The lesions of the upper respiratory tract in these animals were typical of IBR.

None of the vaccinated animals experienced a temperature rise during the period of reaction in the controls. All vaccinated animals were restrained and examined on the fourth and sixth day after challenge. There were small areas of white fibrinous exudate on the nasal septum in most heifers. This has been characteristic of the author's previous experience in challenging immune animals by the nasal spray method. It probably represents a local infection caused by massive application of the inoculum under pressure. No other changes were noted. Two nasal swabs taken from three vaccinates on the third day after challenge yielded the virus when inoculated on tissue culture.
DISCUSSION

Solid protection against IBR was obtained with a single intramuscular injection of 2 ml. of the vaccine. While the exact mode of transmission in naturally occurring outbreaks is not known it is believed that the experimental exposure was more severe than a naturally occurring exposure. The spray application not only forced the virus against the mucous membrane under pressure but the vaporizer fluid containing the virus was inhaled by the animal. In two out of three animals virus was present in the nostrils three days after exposure. The vaccinates were again exposed by being in contact with the controls. The morbidity rate in our group of 35 was 43 percent, which is higher than the rate reported in field outbreaks.

The 100 percent susceptibility of the contact control group to challenge indicated that there is no danger of infection occurring in animals in contact with vaccinated animals.

The appearance of temperature rises of idiopathic origin makes it difficult to assess accurately the effect of vaccination upon the animals. However, regardless of the cause of the temperature rises the effect upon the animal was slight and in most cases these febrile animals were recognized only when the temperature was taken.

This trial was designed to determine if the vaccine when used under field conditions would confer immunity without causing disease breaks in the vaccinates or in animals in contact with the vaccinates. In all categories the vaccine proved satisfactory.

ACKNOWLEDGEMENT

Acknowledgement is made to Miss Midori Wada and Mr. Joseph Saito of the technical staff of the School of Veterinary Medicine for their willing and careful performance of their assignments in this experiment.

REFERENCES

COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


The virtual eradication of tuberculosis and the possible eventual eradication of brucellosis, the two important chronic diseases of cattle in this country, should lend increased interest towards control and research on other equally important diseases.

Bovine mastitis, which may occur as an acute or chronic disease and which has many causes, including the specific bacterial cause, Streptococcus agalactiae, is one of the most important diseases of dairy cattle. It is said to cause an annual loss of fifty million dollars per year in Wisconsin alone. Volumes have been written on mastitis, with many questions still remaining unanswered. There are sufficient existing facts to warrant an optimistic view regarding the control but not the eradication of this disease.

In a few states, notably California, Connecticut, and New York, it has been shown that large numbers of herds may be completely freed of S. agalactiae infection and of mastitis due to this infection. Mastitis due to other infections and to environmental causes remains a constant threat in all herds. The cows affected by the disease have been reduced from an average of 15 percent to 7 percent in herds under careful supervision in New York State. This was true whether or not all of the S. agalactiae infection was eliminated so long as this specific infection was reduced to a point where very few quarters were infected. There have been problem herds where S. agalactiae was essentially impossible to remove from the udders even after several years of clinical and bacteriological study and work. In general, however, frequent bacteriological examinations and frequent treatments have completely freed herds of S. agalactiae within a period of six months or less. It was essential for the owner to cooperate on preventive measures and for the veterinarian to treat effectively and promptly after a laboratory diagnosis was made. All cows with infected quarters that had been injured so that they leaked badly had to be eliminated or be given special treatment using plugs taped in the teats to retain the antibiotics.

It was shown by Hale in Connecticut and by others in England that quarters which are freed of S. agalactiae infection produce 25 percent more milk. This is sufficient reason for promoting programs of mastitis control that include the use of the modified Camp test as described by Murphy and others as a means of making an accurate diagnosis of this and other infections. The physical examination to determine all clinical cases regardless of cause requires the skill of a veterinarian but is essential in an eradication or control effort. All quarters giving abnormal milk are treated on dry and lactating cows. The substitution of the
C.M.T. (California Mastitis Test), known as the paddle test, may prove to be a useful diagnostic aid.

Research is needed on a much more elaborate scale in states with large dairy cattle populations in order to find better methods of control for this specific infection, but more particularly in order to discover the basic facts regarding the prevention of the other infections. At present, we rely in New York State upon prevention more than treatment. This includes control of the mechanical milker from the viewpoint of mechanical efficiency as pointed out by members of our program and by Schalm and others in California. The cleanliness of the rubber parts of the machine and general stable construction and sanitation are important also.

We feel that it is a more logical approach, when we use expensive laboratory diagnostic methods and antibiotic treatments, to employ at all times every known method of preventing teat injury and infection. The milking machine improperly used can cause serious injury. It behooves veterinarians engaged in mastitis control work to become familiar with the detection of plugged air lines, weak pumps, and many other things that go wrong with milking machines.

Specific preventive measures that should be put in effect in all dairy herds whether or not a laboratory diagnosis is available are well described and include proper attention to additions, dipping the teat ends in a suitable antiseptic after each milking, and washing the hands of the milkers or strippers between cows. In England they even suggest the use of penicillin ointment or cream on the hands of the milker to prevent the spread of *S. agalactiae* infection.

**Vibriosis**

The cattle industry has suffered greatly from breeding failures long before artificial breeding became common, but a definite increase in the sterility problem occurred concurrently with the widespread use of artificial breeding. Admittedly, beef and dairy cattle were bringing very high prices at this same period and many bulls, as well as females, were moved from one herd or state to another through sales during the years after World War II. The discovery at this time by Plastridge and others that *Vibrio fetus* was an important venereal pathogen causing a widespread disease, known as vibriosis, marked a milestone in the control of genital diseases. McEntee, Hughes and Gilman developed a sound technique several years ago for complete control of vibriosis in all beef and dairy herds when artificial breeding was employed exclusively. They suggested the use of 500 to 1,000 units of crystalline penicillin and of 500 to 1,000 micrograms of dihydrostreptomycin per ml. of diluted semen. This is a plan of control of vibriosis that has proved to be successful and should be more widely used. This special antibiotic-treated extender, 25 to 30 parts, and semen, one part, should be allowed to cool from body temperature to 40°F. during a six-hour period before it is placed in the cervix. The same treatment should be applied to semen that is to be frozen, but proof is needed to show that it works as well with frozen semen. The glycerol may have the ability at times to revive the stunned *V. fetus* as well as to preserve the life of the sperm cell.

Many other causes of bovine sterility are treated by use of specific hormonal
and other therapy with success, but in the vast majority of problem herds, it is as essential to eliminate all sources of vibriosis as it is to eradicate brucellosis. This is especially true if breeding stock is being sold, since infected bulls or females added to clean herds can readily spread the disease where direct service is employed.

**VIRUS DISEASES AND OTHER DISEASES**

Other important infectious diseases, or diseases suspected of being due to viral or other closely related agents, such as virus diarrhea and virus diarrhea-like diseases, may have an indirect bearing upon reproductive efficiency. Most acute infections, especially viral agents, cause occasional abortion in cattle at all stages of pregnancy. It is well known that there are other more important losses from these viral diseases.

The disease called shipping fever is still a very important problem in most areas. The etiology of the disease is complex, possibly including an undiscovered virus. It is complicated by parasitism and environmental changes. Success has followed the segregation of the affected animals and preventative treatment of the unaffected cattle with certain sulfa preparations. Symptomatic treatment, including the use of various antibiotics and blood transfusions, gives favorable results in a fair percentage of the affected animals. An immunizing agent that would regularly decrease the losses from this condition called shipping fever would be welcome.

Foot rot (interdigital necrosis) with the usual complications causes extreme financial losses among dairy herds and range cattle. A second disease having a fetid ulcerated skin in the interdigital space and secondary pododermatitis is very common in all types of stables in the eastern dairy cattle areas. The etiology of both diseases deserves study. Prevention by copper sulfate solution used in foot baths or copper sulfate one part and slaked lime 10 parts in lime boxes are helpful but inadequate even in dairy herds that are stabled daily. Antibiotics, sulfa, and proper hoof trimming assist in the treatment, but an effective agent to prevent the diseases is needed.

Leptospirosis deserves recognition as a disease entity and control measures should be based upon existing and future research.

The most pertinent information regarding the confusing status of infectious bovine rhinotracheitis, virus diarrhea, and so-called mucosal disease has been summarized by Charles J. York of this committee as follows:

Since the report of the Committee on Infectious Diseases of Cattle for 1955, additional information has been worked out concerning the respiratory-mucosal disease-virus diarrhea complex. It is now well established that infectious bovine rhinotracheitis (IBR) is caused by a specific virus, distinct from the etiological agent, or agents, of mucosal disease or virus diarrhea. This IBR virus has been propagated by tissue culture procedures, and the tissue culture virus used successfully to reproduce the disease experimentally as seen in naturally occurring cases. Furthermore, by the use of a serum neutralization procedure, a serological test has been developed which is a valuable aid in experimental studies, in identification of disease outbreaks, and in epidemiological surveys concerning IBR disease. With the discovery that this virus can be grown by tissue culture procedures, the development of a practical vaccine for the control of this disease is a step which will probably be completed in the near
future. As a result of adequate descriptions of this disease by various investigators, as well as the development of a serological test, infectious bovine rhinotracheitis has now been observed in at least 15 states. With the apparent widespread occurrence of this infection, it seems probable that it is only a matter of time before the disease will be detected in other areas.

Although there are a number of very marked differences between infectious bovine rhinotracheitis and virus diarrhea, there may be at times outbreaks of virus diarrhea where, in the absence of well-developed mouth lesions or diarrhea, confusion might arise as to the exact diagnosis of the infection in the herd. A serological test for virus diarrhea is urgently needed, but until such time as this test is developed, the employment of the IBR serological test will be of value as an aid in differential diagnosis.

It has recently been demonstrated that virus diarrhea as described in New York State occurs in the Midwest, although information at the moment indicates that at least one other virus, producing similar clinical signs of illness, occurs in the same general area. It has also been found that mucosal disease is due to an infectious agent which can be transferred experimentally from animal to animal. The relationship and importance of each of these etiological agents has not yet been worked out, but with a number of research programs now under way in various experimental laboratories, it may be possible to present a much clearer picture of these diseases by this time next year. Although the exact diagnosis may at times be in doubt, mucosal disease or virus diarrhea in one form or another have been reported as occurring in at least 24 states in 1956, indicating a wide distribution of these diseases in this country. It is also of interest to note that a disease of cattle resembling the virus diarrhea-mucosal disease complex has recently been reported in England, suggesting that the incidence of this type of condition may be more extensive than previously suspected.
STATUS OF FEDERAL-STATE COOPERATIVE TUBERCULOSIS ERADICATION

A. F. Ranney

During the fiscal year 1956, some 9,220,000 cattle were tested for tuberculosis and 14,363 reactors were found. This amounted to 0.15 percent of reactors among the animals tested. For the second successive year there has been a slight increase in the percent of reactors found over the previous year. It may be recalled that the percentage of reactors remained at 0.11 for each of the three fiscal years prior to 1955 when the rate was shown at 0.12 percent. We should anticipate moderate increases in percentage of reactors based on tests made during the next few years if we foster the type of program that will most effectively result in seeking out and removing from our herds, animals affected with bovine tuberculosis.

One of the well-known problems connected with tuberculosis eradication is the reactor showing no visible lesions. Speaking on that subject to this organization in 1947, Dr. Howard Johnson had this to say:

We must keep foremost in our minds that our chief aim is to eradicate bovine tuberculosis. To accomplish this end, no reacting animal should be left in a known tuberculous herd.

This will mean that NVL cases will be seen at the time of slaughter. However, it is well known that early lesions of tuberculosis are very difficult to detect even on microscopic examination and also that it is impossible to examine all parts of an animal at postmortem. Therefore, the fact that no gross lesions were observed is not proof that the disease was not present. In known infected herds our concern is with the possible tuberculous animal which failed to react, not in the animals in which we failed to demonstrate lesions.

After having talked with a considerable number of people interested in the ultimate eradication of bovine tuberculosis, it seems to be taken for granted that many reactors in herds of unknown status or in supposedly non-tuberculous herds are not removed when they are found. They are considered to be ‘deviators’ and as such are ignored in the records. If cattle are classed as deviators and not reported, all calculations on reactors are undependable. In this connection the chances of a ‘deviator’s’ being tuberculous are probably as great as that of a reactor being tuberculous. In other words, if 60 tuberculous animals are found in 100 reactors (which is nearly the present percentage), 100 deviators might furnish an equal number of infected animals. The probabilities could be calculated if sufficient material were available. Results indicate a high incidence of tuberculosis among some of the so called ‘deviators’.

Dr. Asa Winter, reporting to this Association in 1949, made this contribution to our thinking on the NVL problem:

One of the much discussed questions in connection with this program has been the no-visible-lesion reactor. Since those factors responsible for non-specific reactions tend to remain fairly constant, the reverse ratio of no-visible-lesion cases to total re-

3Dr. A. F. Ranney, Chief, Tuberculosis Eradication Section, Animal Disease Eradication Branch, Agricultural Research Service, United States Department of Agriculture.
actors is a condition that must be expected with the decline in rate of infection. This has become an increasingly sensitive subject and one that demands continued study.

While the no-visible-lesion reactor presents an embarrassing and costly problem, with research being directed toward its solution, the question of locating remnant sources of exposure is of much greater importance as a disease eradication factor.

Very little research is presently being directed toward a solution to this problem. Figure 1 shows graphically how the number of carcasses condemned because of tuberculosis has declined since 1927. It also depicts how the ratio of NVL cases to the number of cattle tested remained rather steady until the country approached a modified accredited status in 1940 while the ratio of NVL cases to reactors slaughtered has increased steadily as the incidence of infection has been reduced.

In our report to this Association a year ago, we called attention to the importance of three activities which, if properly carried out, should materially improve the effectiveness of the tuberculosis eradication program. They are:

1. Maintaining strict quarantine of infected herds.
2. Obtaining the history of each reactor. If there is even a remote possibility that a reactor may have brought tuberculosis into a herd, the herd of origin should be determined without fail, and contact animals should be tested.
3. Insuring that all animals believed to have been exposed are satisfactorily accounted for. Those that have not been slaughtered should be tested.

These are only three of several important considerations that require attention. When the tuberculosis eradication program was much younger, we meas-
TABLE I
**Tuberculosis Eradication Tests**
January 1–June 1, 1956

<table>
<thead>
<tr>
<th>Reason for Test</th>
<th>Cattle Tested</th>
<th>Reactor Found</th>
<th>Reactor Cattle</th>
<th>Tests Conducted</th>
<th>Reactors Disclosed</th>
<th>Cattle Tested to Find One Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area Retest</strong></td>
<td>3,920,137</td>
<td>5,063</td>
<td>0.13</td>
<td>73.9</td>
<td>62.0</td>
<td>774</td>
</tr>
<tr>
<td>Test of Herd for Accreditation or Reaccreditation</td>
<td>613,590</td>
<td>221</td>
<td>0.04</td>
<td>11.5</td>
<td>2.7</td>
<td>2,776</td>
</tr>
<tr>
<td>Test for Sale or Show</td>
<td>118,686</td>
<td>32</td>
<td>0.03</td>
<td>2.2</td>
<td>0.4</td>
<td>3,709</td>
</tr>
<tr>
<td>Test for Interstate Shipment</td>
<td>68,725</td>
<td>3</td>
<td>0.004</td>
<td>1.3</td>
<td>0.1</td>
<td>22,908</td>
</tr>
<tr>
<td>Test of Imported Animals (Interstate or Foreign)</td>
<td>24,546</td>
<td>29</td>
<td>0.12</td>
<td>0.5</td>
<td>0.3</td>
<td>846</td>
</tr>
<tr>
<td>Retest of Herd under Quarantine</td>
<td>176,893</td>
<td>1,939</td>
<td>1.10</td>
<td>3.3</td>
<td>23.3</td>
<td>91</td>
</tr>
<tr>
<td>Test After Tracing Animals with Lesions on Regular Kill (ADE 6-35)</td>
<td>24,938</td>
<td>390</td>
<td>1.56</td>
<td>0.5</td>
<td>4.8</td>
<td>64</td>
</tr>
<tr>
<td>Test After Tracing Reacting Animals</td>
<td>1,892</td>
<td>21</td>
<td>1.11</td>
<td>0.1</td>
<td>0.3</td>
<td>90</td>
</tr>
<tr>
<td>Test After Tracing Exposed Animals from Infected Herds</td>
<td>10,602</td>
<td>104</td>
<td>0.98</td>
<td>0.2</td>
<td>1.3</td>
<td>102</td>
</tr>
<tr>
<td>Other</td>
<td>342,649</td>
<td>356</td>
<td>0.15</td>
<td>6.5</td>
<td>4.3</td>
<td>962</td>
</tr>
<tr>
<td><strong>U. S. Totals</strong></td>
<td>5,302,658</td>
<td>8,158</td>
<td>0.15</td>
<td>100.0</td>
<td>100.0</td>
<td>650</td>
</tr>
</tbody>
</table>

ured progress by the number of counties that reached the goal of modified accredited status. Today, our progress is less spectacular as we seek out much rarer cases of the disease and attempt to wipe out infection in each area where it is found. This is not only of local importance—in this day of prodigious livestock movement, eradication of disease in one place protects many others that might receive replacement animals from it.

Data assembled over a six-month period during the past fiscal year, presented in Table 1, show that in the course of area retests we found 62 percent of the total reactors among 73.9 percent of the cattle tested. On the other hand, we found in quarantined herds almost one-fourth of all the reactors by applying tests to only 3.3 percent of the animals tested during the same period. The table also shows that one reactor was found in quarantined herds for every 91 animals tested, as compared with one reactor for every 774 animals tested to reaccredit areas. These figures clearly point up the fact that, as a minimum procedure, infected herds should be kept under strict quarantine until enough retesting has been done to declare the herd tuberculosis-free. Permitting known exposed animals, without proper retests, to enter the channels of trade except for immediate slaughter cannot be justified.

It is obvious that the provisions of the Uniform Methods and Rules, adopted by this Association and approved by the Agricultural Research Service, are not
being fully complied with in all states. Several counties in a few states are overdue for reaccreditation. It is imperative that delinquent counties be brought up to date in accordance with the accepted standards. Otherwise, approved procedures will certainly fail to merit confidence and respect.

In at least two states, herds are not quarantined when reactors are found. In three states, herds are routinely maintained under quarantine for only one retest after infection is found. The Uniform Methods and Rules specify a minimum of two negative tests, the first in not less than 60 days after infection was found, the second between five and six months after disclosure of infection.

In four states, herds in which no-visible-lesion reactors have been found are not quarantined for even one retest. In two other states the quarantine is released upon receipt of a necropsy report showing that no visible lesions of tuberculosis were found in the reactors slaughtered.

During the past year, procedures have been inaugurated to report the results of tests made after tracing reacting animals to herds of origin and the follow up on animals that have been exposed. These procedures furnish a basis for a comprehensive compilation of data, studies of which should lead to development of a more effective eradication program. We hope that these studies will shed some light on the relative number of no-visible-lesion reactors that may have had a history of association with known infected animals but are found in herds believed to be tuberculosis-free.

Complaints have been made for several years by states having a relatively low incidence of infection that most of the reactors they find originate in other states. The use of uniform procedures to promptly report all such cases to officials in states from which reacting animals originated should help to pinpoint the specific problems and make it possible to reduce the number of such cases in the future. These procedures appeared rather formidable when first set up, but the general response to this method of compiling data has been on the whole encouraging. We are still looking for improvements in current reporting procedures. Comments received from many sources indicate that a uniform system for reporting the tracing of infected and exposed animals is accepted as an essential part of an efficient eradication program.

Improved methods of tracing to herds of origin animals that have reacted in the regular testing program and those showing lesions on regular kill suggest that the increase in percentage of reactors is due not so much to a greater incidence of the disease as to greater efficiency in finding animals that have infection.

Routine testing, while still very necessary, results in screening a relatively large number of animals to find one reactor as compared to tests made after tracing diseased or exposed animals. Table 1 shows the number of animals tested, for various reasons, to find one reactor. When we get into retesting quarantined herds and tracing actual cases to their source, we are working closer to the disease and we get better results in locating infected animals. When we test after tracing exposed animals from infected herds, we find a reactor in 102 tests; we find one in 91 tests when retesting a herd under quarantine; we find one in 90 tests after tracing reacting animals; and we find one in 64 tests when tracing back from animals found to have lesions on regular kill.
A number of case histories serve to illustrate the value of tracing procedures:

One rather involved case centers around a dealer's herd in Connecticut, where eight reactors were found in May 1955. Five of the eight reactors had originated in Maine, two in Connecticut, and one had been found in the dealer's herd on a previous test. Investigations were made in all herds from which animals that reacted had been consigned to the dealer where infection was found; no reactors were reported in the parent herds. However, by tracing animals that had moved through the dealer's herd during the previous six-month period, and the finding of reactors on routine tests that were identified as having passed through the dealer's herd during the same period, infection was found in three states—seven reactors in four Connecticut herds, seven in three Massachusetts herds, and six in two herds in Rhode Island.

In September 1955, the carcasses of two animals revealed lesions on regular kill in a Massachusetts slaughterhouse. These animals carried Maine ear tags. After considerable investigation it was determined that they were sold from Maine to the Connecticut dealer mentioned above, who sold them to a Massachusetts herd owner who later consigned them to slaughter. As a result of this many-pronged investigation, 20 reactors were located in nine of the 25 herds tested. There were also ten dealers who had handled animals going to or from the dealer's herd that had infection. Subsequent tests turned up two additional reactors in the Connecticut dealer's herd.

In Ohio an owner sold his entire herd of 88 Angus cattle in July 1956, anticipating that all would go for immediate slaughter. The animals were taken to an auction sale where 48 went to 12 different slaughtering establishments. Federal inspectors at one plant and municipal inspectors at two plants reported five carcasses as showing lesions of tuberculosis. Forty of the 88 animals were sold to three farmers and three dealers. Tests on these premises and four additional farms to which dealers had sold some of the cattle revealed 23 reactors on seven premises. One purchaser who had a reactor sold two animals in violation of the state quarantine on his herd, and was placed under $500 bond. This case illustrates how rapidly animals from infected herds can be scattered. It also illustrates how rapidly tracing can be done—from sales of the herd on July 5, only 22 days elapsed before the last animals from the herd had been located and tested. Had not this prompt and thorough tracing been done by cooperating state and Federal officials, the infection from this herd might have been so scattered that an incalculable amount of damage would have resulted. This example also illustrates what may happen when animals are consigned to market for slaughter purposes and the market agencies may find it profitable to divert a portion of the consignment for dairy, breeding or feeding purposes. Similar diversions may occur when animals are consigned for slaughter from quarantined herds unless preventive measures are employed.

Another Ohio cattle owner operated a tuberculosis-free dairy before 1954, when he sold all his cattle but three. In the fall of 1955 he bought some feeder cattle and about eight months later began marketing a few of these animals. Lesions of tuberculosis were found in two of them at a packing plant in a neighboring state, and lesions in swine from the same farm were reported from a local packing plant. Within a few days, 22 cattle, 14 hogs, and 28 chickens on his farm were given tuberculin tests. The entire herd of cattle and ten of the chickens reacted to the test; the hogs tested negative. Nineteen of the 22 bovine reactors showed lesions of tuberculosis on post-mortem examination. There was no indication that the infection had spread to livestock on other premises. While infection was not found on other premises as a direct result of these tracing procedures it does alert us to the fact that we must not overlook feeder cattle as a possible foci of infection.
The efficiency of tracing procedures and the results obtained would be materially bettered if it were possible to have each animal going to slaughter fully identified and those showing evidence of disease reported to livestock sanitary officials by this identification. The advantages of full identification were well illustrated during fiscal 1956 in handling brucellosis-infected animals that showed lesions of tuberculosis when slaughtered.

This situation occurred in 39 cases. Each of these animals was thoroughly identified by brucellosis reactor tags as required by the brucellosis eradication program. Because of identification, it was possible to trace 100 percent of these lesion cases speedily to their herds of origin. Compared with this perfect record of tracing, it was impossible to trace 88 of the 495 lesion cases reported by Federal meat inspectors. Failures in tracing were primarily due to lack of any identification in some cases and very scanty descriptions in others.

Some state and local organizations are taking steps to provide for better identification of animals. A program was initiated at the Buffalo, N. Y. Stockyards in February 1955 to identify cattle consigned for slaughter purposes. This was accomplished by recording the eartag number of each adult dairy animal with the name and address of the consignor. During the first year of operation, four tuberculous animals were reported by veterinary meat inspectors in packing plants that had purchased cattle from the Buffalo market. In each case the origin of the animal had been recorded, and within a few hours arrangements were made to test the herd of origin. The result—24 reactors were removed from three herds on the first tests following the tracing.

It was estimated that about three-fourths of one employee's time was required to obtain the records of cattle identification. This fraction of his pay amounted to about $2,931 to which $774 was added to cover cost of testing the herds involved, making an estimated outlay of $3,705. A very conservative estimate of the cost of locating one reactor in the course of routine testing in New York State during 1953 was $738.97, while locating reactors in herds, to which lesions cases were traced, cost approximately $32.25 per reactor.

Figured on this basis, it would have cost $17,735.58 to locate 24 reactors, as compared with $3,705. Thus, according to conservative estimates, the cattle identification program at the Buffalo market in its first year yielded a saving of more than $14,000.

Tracing techniques have reached a high degree of ingenuity and efficiency in some states. As they approach maximum efficiency in all states, it is fair to predict that statistics on reactors found will again turn downward, reflecting a scarcity of tuberculosis.

Again I should like to refer to Doctor Johnson's paper in which he states:

A considerable reservoir of infection will remain if the diseased animals are not detected. Whether the disease will eventually be eradicated will apparently depend upon whether infected animals are detected faster than animals become infected. That in turn may depend upon the number tested per year and also upon whether the animals tested are either a group with average infection or a group with more than average infection.

How many cattle should be tested annually to maintain the status quo? That is,
to detect animals as fast as they become infected. Calculations made by a statistician and based on figures contained in the 1946 issue of Agricultural Statistics indicate that the eight and nine million cattle that have been tested annually in recent years are not quite sufficient to control the disease even if it is assumed that the disease is confined to dairy herds.

We are now testing annually about a million more animals than were being tested in 1946. The cattle population, however, has increased from 82.2 million in 1946 to 97.4 million in 1956. The ratio of cattle tested to cattle population is not quite as favorable now as it was ten years ago.

Tuberculosis eradication is not now and has never been easy. We should continue to tighten our grip on bovine tuberculosis whenever and wherever it may be suspected or found. The tubercle bacillus is a redoubtable enemy. It should be fought with our best thinking, our best practices, and all the ingenuity we can apply to the problem.
PATHOLOGIC STUDIES ON TUBERCULIN REACTORS WITH NO VISIBLE LESIONS*


In the campaign for the control of tuberculosis in cattle, it is generally recognized that the problem of the tuberculin reactor, in which no recognizable lesions of tuberculosis can be demonstrated at autopsy, is of major importance. A variety of reasons have been advanced to explain the phenomenon of an allergic response to tuberculin in the absence of visible glandular or visceral lesions of tuberculosis. Crawford (1), in his discussion of this subject mentions the following factors:

1. Sensitization to avian or human tubercle bacilli.
2. Tuberculoid skin lesions.
3. Tuberculous lesions which are either occult or too minute to be readily observed macroscopically.
4. Acid-fast organisms other than Mycobacterium tuberculosis that may be capable of setting up a sensitization to tuberculin in bovine animals without producing apparent lesions.

In addition, Groth (2) and Johnson et al. (3) have shown that of cattle sensitized to Mycobacterium paratuberculosis, one-third may be expected to react to the tuberculin test.

As a part of a recent epidemiological investigation, which was made in an area where the problem of no-visible-lesion reactors has been particularly prominent, an opportunity was afforded to make a laboratory examination of specimens of intestine and mesenteric lymph nodes from 51 cattle which had reacted to the tuberculin test. Available autopsy reports (4) indicated that of these 51 reactors, seven showed skin lesions, 2 showed teat lesions, and four had minute, calcified foci in one or more lymph nodes. In the remaining 38, no gross granulomatous lesions, suggestive of a tuberculous process, were detected.

The purpose of this study was to examine the intestinal mucosa and regional lymph nodes for microscopic evidence which might suggest a reason for sensitization to the tuberculin test. It was particularly desired to determine whether acid-fast organisms could be demonstrated in an appreciable number of lymph nodes which showed no grossly detectable lesions.

MATERIALS AND METHODS

In each of the 51 cases, the tissues to be examined consisted of approximately 24 square inches of large and small intestines, cut to include the ileocecal valve. In addition, two or three mesenteric lymph nodes were submitted from each ani-

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mal. These had apparently been chosen at random since, with two exceptions, no granulomatous lesions were exposed. All tissues were preserved in borax for shipment. The lymph nodes were fixed in 10 percent formalin solution upon receipt at the laboratory.

Stained smears were prepared from the mucosa of the intestines at various levels and examined microscopically for the presence of acid-fast bacilli, morphologically characteristic of *M. paratuberculosis*.

Multiple cuts at close intervals were made through the mesenteric lymph nodes, and a careful examination was made for evidence of minute focal lesions. In each case, four blocks were sectioned to represent a complete cross section of two lymph nodes. Care was taken that any grossly visible suspicious lesions were included. Duplicate sections were routinely cut at six microns and stained by the hematoxylin-eosin and carbolfuchsin-methylene blue methods, respectively. Examination of the hematoxylin-eosin stained sections was made for evidence of a cellular reaction, and in all sections, regardless of whether inflammatory changes were found, a careful search was made for the presence of acid-fast bacilli.

**RESULTS**

Small acid-fast bacilli, morphologically characteristic of *M. paratuberculosis*, were found in stained smears of the intestinal mucosa from four cases. Sections of the mesenteric lymph nodes from two of these cases showed numerous acid-fast bacilli, together with a diffuse granulomatous reaction made up, chiefly, of epithelioid cells and numerous giant cells (fig. 1). The lymph node pathology was considered to be characteristic of that usually associated with *M. paratuberculosis* infection.

![Fig. 1. Johne's disease, mesenteric lymph node. Diffuse granulomatous reaction consisting of epithelioid cells and giant cells. Hematoxylin-eosin. \( \times 90 \).](image)
In addition to the above-mentioned lymph node lesions of Johne's disease, acid-fast organisms were demonstrated in five additional cases among the 51 specimens of mesenteric lymph nodes examined. In four of these, focal granulomatous changes were found.

**Case 133546** (fig. 2). One node contained three barely visible calcified foci. Similar lesions were noted in other nodes at autopsy. Microscopically, these appeared as partially encapsulated caseocalcareous centers surrounded by a considerable epithelioid reaction and occasional giant cells. Acid-fast bacilli were found within the reactive areas.

**Case 133557** (fig. 3). One node contained a single encapsulated calcified lesion the size of a pinhead. The other node contained three smaller calcified centers. These were grossly visible but could have been readily missed. Histologic sections showed the lesions to be well-encapsulated granulomas made up of caseocalcareous centers, a pronounced epithelioid reaction, and numerous giant cells. Acid-fast bacilli were readily demonstrated.

**Case 133561** (fig. 4). A single calcified focus, barely visible to the naked eye, was located in the hilus of one node. The lesion was not noticed on gross examination, but calcium was evident when the sections were cut. This proved to be an encapsulated abscess filled with partially calcified debris and numerous polymorphonuclear leucocytes. There was no accompanying epithelioid reaction, and giant cells were not apparent. Acid-fast bacilli were found within the necrotic center.

**Case 133549** (fig. 5). No lesions were reported at autopsy, nor were any grossly apparent in the mesenteric lymph nodes examined. Examination of histologic sections revealed several microscopic, circumscribed, granulomatous areas within the lymph node parenchyma. These were made up of focal aggregates of polymorphonuclear leucocytes interspersed among disintegrating small round cells. Encapsu-
lination was indefinite. Occasional acid-fast bacilli were encountered, but not all were confined to the reactive area. These, in our opinion, were actually occult lesions which could not have been noted at autopsy.

*Case 133564.* No changes were observed at autopsy in this animal, nor were any demonstrated in the lymph node specimen. Microscopically, there was no evidence

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**Fig. 3.** Two encapsulated granulomatous foci containing acid-fast bacilli in a mesenteric lymph node. Hematoxylin-eosin. ×8.

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**Fig. 4.** Single encapsulated abscess (arrow) containing acid-fast bacilli in the hilus of a mesenteric lymph node. Hematoxylin-eosin. ×4.
Fig. 5. Occult granulomatous lesion containing acid-fast bacilli in a mesenteric lymph node. Hematoxylin-eosin. ×40.

of a cellular reaction, but four acid-fast bacilli were found in sections of one node. These were lying free in an apparently normal parenchyma.

Since no acid-fast bacilli were found in the intestinal mucosa in this case, it is believed probable that this represents invasion of a mesenteric lymph node by organisms other than *M. paratuberculosis*. However, despite the absence of a cellular reaction the finding of acid-fast bacilli within a lymph node cannot be disregarded and their presence suggests a possible explanation for sensitization to tuberculin in this instance.

Miscellaneous changes encountered in this survey included three cases of parasitic granuloma, characterized by focal accumulations of eosinophils, many of which were disintegrating. One well-encapsulated granuloma, the size of a match head, proved to be actinobacillosis. In three other lymph node specimens, small deposits of calcium were encountered. These were not accompanied by a cellular reaction, and careful search failed to reveal the presence of acid-fast organisms. An accumulation of iron-staining pigment, which was grossly visible as a brown discoloration, was a common finding.

**DISCUSSION**

The findings of this study are not to be considered typical of those that might be obtained from a study of all so-called no-visible-lesion reactors since there is no assurance that the 51 cases examined were a representative sample. In this instance, however, the detection of skin lesions in seven cases, teat lesions in two, intestinal and lymph node lesions of Johne's disease in four, and the demonstration of acid-fast organisms in the mesenteric lymph nodes of five other animals suggests a possible reason for reaction to the tuberculin test in 18 cases. The lymph
node specimens represented only a small proportion of the mesenteric chain, and it is possible that, had it been feasible to make a more complete histologic examination, additional microscopic lesions, containing acid-fast organisms, might have been discovered.

One of the primary objectives of this investigation was to determine whether an appreciable number of the lymph nodes examined would contain acid-fast organisms in the absence of visible lesions, thus suggesting a possible reason for sensitization to tuberculin. This was not the case. In only one instance were acid-fast bacilli demonstrated in the absence of either a granulomatous reaction or accompanying evidence of \textit{M. paratuberculosis} in the intestinal mucosa. Only one occult granulomatous lesion was demonstrated. The lymph node lesions in the other three cases were grossly visible but were of such small size that they might have been readily overlooked at autopsy. In this connection, it must be realized that, in the abattoir, practical limitations of time and personnel frequently prohibit the exhaustive search for minute lesions which might be made by an operator who had unlimited time at his disposal.

In any event, there was nothing to distinguish these granulomas, caused by acid-fast organisms, from others having a different etiology. In a study of suspected tuberculous lesions from 35 reactor cattle, Davis and Anderson (5) found 35 percent of the lesions to represent conditions other than tuberculosis. This indicates the value of a laboratory examination, in addition to a thorough autopsy, as an aid in minimizing the number of tuberculin reactors which must be reported as having shown no visible lesions at autopsy.

\textbf{SUMMARY}

A report is made of the pathologic findings in the intestine and mesenteric lymph nodes of 51 cattle which had reacted to the tuberculin test but which showed no characteristic visceral or glandular lesions of tuberculosis at autopsy. Evidence of Johnes disease was found in four cases. Acid-fast bacilli were demonstrated in the mesenteric lymph nodes of five additional cases. Of these, three were associated with pinhead-size caseocalcareous foci and one with an occult lesion. Acid-fast bacilli, in the absence of a cellular reaction, were demonstrated in the lymph nodes of only one case.

\textbf{ACKNOWLEDGMENT}

The authors wish to extend acknowledgment to Dr. W. C. Logan, Animal Disease Eradication Branch, Agricultural Research Service, Urbana, Illinois, for information concerning the autopsy findings.

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REPORT OF THE COMMITTEE ON TUBERCULOSIS


Your Committee on Tuberculosis would like to call your attention to the report of the 1946 Committee from which we quote:

"In previous reports your Committee on Tuberculosis has called attention to the dangers arising from a too-complacent attitude toward this disease. In the minds of too many livestock men, tuberculosis is regarded as a thing of the past; as a bad problem that has been licked and can now be forgotten.

This group does not need to be told that this problem has not been finished; that tuberculosis in cattle has not been completely licked. It is true that a magnificent job was done during the twenties and thirties, a job that has been widely acclaimed both here and abroad. During this period, with no serious disruption of the cattle industry, with no serious shortages of dairy products, the greater part of the tuberculous animals were discovered and eliminated. Most of our herds were freed from this disease and have remained free since that time. But the disease has not been eradicated, and that was, and is, our goal. So long as even a few infected animals remain, a menace exists which could, within a few years, put us back where we started in 1917."

These and other points outlined in the report for the 1946 Committee are of no less significance today than they were 10 years ago when the report was written.

Your Committee would like to submit the following recommendations for consideration by this Association:

1. That action on the proposed changes to the uniform methods and rules for tuberculosis eradication considered by this Association last year be held over for another year for additional study and consideration. During the interim, the uniform methods and rules for the establishment and maintenance of tuberculosis-free accredited herds of cattle and modified accredited areas adopted by this Association in 1953 should remain in effect.

2. That the regional groups of the United States Live Stock Sanitary Association at their regular meeting during the coming year provide for sufficient time on their program to thoroughly review the status of tuberculosis eradication in their respective regions.

3. That the cooperative State and Federal Live Stock Sanitary Officials and other co-operating agencies in each State promptly review the procedures and policies now in effect in an effort to strengthen the tuberculosis eradication program.

4. 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.
5. 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.

6. That steps be taken in all States where counties are overdue for reaccreditation to bring all counties up to full accreditation status in accordance with the uniform methods and rules for tuberculosis eradication.

7. That State Officials in States that are experiencing difficulty in obtaining an adequate appropriation to conduct the tuberculosis eradication program in a manner that will enable them to comply with the uniform methods and rules advocate to the responsible parties that necessary funds be appropriated at the next session of their legislature to enable them to properly conduct their program.
WHAT PRECAUTIONS ARE TAKEN TO PREVENT INTRODUCTION OF FOREIGN DISEASES

F. L. HERCHENROEDER, D.V.M.*

Fort Worth, Texas

In the days before the advent of the airplane and the fast-moving ship, the introduction of disease by animals or products was not of quite the same moment as it is today. Prior to 1850 the movement of livestock was confined to those animals which would accompany the pioneer as he moved across the country and spaces were so vast that the possibility of the spread of infection was slight. In order to give you an insight as to how disease was introduced into the United States, I would like to tell a story which actually happened.

About 100 years ago, a British vessel with the name of George Washington docked in the port of New York, almost opposite the window of our office at 45 Broadway. On this ship were some cattle which had been brought along to furnish milk and food for the crew members and passengers. The particular cow that furnished the milk had gone dry so that the master of the vessel proceeded to take the cow off the ship to a rural district then known as “The Bowery” where he traded this cow for a fresh cow. This transaction being done he returned to the ship and sailed away. The cow he traded, however, was infected with contagious pleuropneumonia which spread over the United States, particularly the eastern seaboard and as far west as Ohio, and for a number of years this particular disease was the scourge of the infant livestock industry in the United States.

Luckily, even in those days, there were people farsighted enough to consider the eradication of contagious pleuropneumonia of grave importance and in about six or seven years after the program was started, this disease was eradicated from the United States. The introduction of this particular disease into the United States in this manner is a situation which could not occur under our present laws and regulations.

In 1884, the Congress established what was formerly known as the Bureau of Animal Industry and provided for certain quarantine stations to be established at the several ports of entry to guard against just such an occurrence. Since that time, our work has taken on considerable more activity in that we are not alone concerned with the introduction of live animals from known foot-and-mouth disease or rinderpest infected countries but also with the possibility of the introduction of such diseases through the importation of animal by-products, hay and straw packing material, garbage, etc.

You have heard of the danger of foot-and-mouth, rinderpest and these other exotic diseases and the grave situation this country is placed in in trying to keep these diseases out—what do we do about it?

First in importance, I would class ships garbage and meat from passenger

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baggage. There has been an outbreak of foot-and-mouth disease directly traceable to garbage from a ship.

Since the law, section 306 (a) of the Tariff Act of 1930, has been in force we have controlled the movement of garbage, meats and animals from ships in the following manner:

All ships entering our territorial waters, at Public Health quarantine must declare whether they are carrying ships stores of meats from foot-and-mouth disease or rinderpest countries, or ruminants or swine from such infected countries. In case animals are aboard, AIQ is immediately notified and drastic action is taken to see that these animals are slaughtered and disposed of in such manner as to eliminate any danger of exposure to our livestock.

No garbage from ships carrying foreign meats may be brought off a ship unless it is in a tight container and brought off for incineration under the supervision of an AIQ inspector.

Hides and skins from infected countries are allowed entry into this country only if the skins are hard-dried, having the appearance of parchment or pickled in mineral acid, or if they are brought into the country for restricted handling in establishments approved for the handling of restricted products where the product is thoroughly disinfected by the use of a sodium bifluoride disinfectant for a 24-hour period.

Wool is allowed entry if free from blood stain and reasonably free from animal filth or dirt.

Bristles are allowed entry if cleaned, washed, and sorted before coming to the United States.

Fresh, chilled, or frozen beef, veal, lamb, and pork are prohibited entry into the United States from infected countries. Cured or cooked meats of these classes may be admitted to this country if thoroughly cured or cooked, and brought into the country to plants where it is to be processed before being sold to the trade.

Hay and straw packing material is prohibited from infected countries unless such hay and straw is disinfected in such a manner as to destroy the rinderpest and foot-and-mouth disease virus. This may be by use of live steam and form-aldehyde.

Animal glands are the only fresh products allowed entry from a foot-and-mouth disease or rinderpest infected country, these to be brought in and processed for pharmaceutical purposes only. A great deal of care must be used in the handling of such products. They are sent to approved establishments where the entire process of manufacture is adequate to guarantee a disease-free final product.

Animal stomachs and rennets are allowed entry if dried to the consistency of parchment or may be entered as restricted products in establishments where the product is handled in such manner as to prevent the introduction of foot-and-mouth disease and rinderpest.

Bone meal, hoof and horn meal, meat meal and tankage. Bone meal for feed or fertilizer must be a steamed or degelatinized product with a nitrogen content of 2 percent or less. Hoof and horn meal may be imported if it has been heated to a degree of heat to guarantee freedom from foot-and-mouth disease, rinder-
pest and anthrax. Tankage and meat scrap may be imported if accompanied by a certificate showing it has been heated to a temperature to destroy foot-and-mouth disease and rinderpest virus and to contain 35 percent BPL or less.

Domestic cattle, sheep, and swine are prohibited entry into the United States from any country where foot-and-mouth disease or rinderpest exists.

All classes of animals, ruminants, swine, and poultry, except horse stock, can come to the United States (except from Mexico and Canada) only after a prior permit has been secured for such entry. When a permit is issued, certain requirements are set up, such as 60 days at the port of export, veterinary certification by government veterinarians of the country of origin, including certain diagnostic tests. After these requirements have been met, the animals are allowed entry into our ports. Before being unloaded from the plane or ship, they are given a complete veterinary inspection by our port veterinarian and if everything is in order, allowed to move forward to quarantine and such tests as are required there.

Horses are inspected on the carrier on arrival and if they come from certain countries, are held in quarantine for a blood test for trypanosomiasis and glanders.

Wild ruminants may be permitted entry provided they are destined to an approved zoo where they can be held in strict control and not be moved about the country.

Since the recognition of swine fever in Africa, wart hogs and bush pigs for zoological parks have not been permitted entry.

Biologics, vaccines, serums and various hormone and glandular substances, all are suspect from rinderpest or foot-and-mouth disease countries and are held under control until thoroughly investigated as to whether it is safe to permit their entry.

In this day and age of seeking additional knowledge, we are constantly requested to allow the entry of disease cultures, vectors, blood samples, for educational institutions and laboratories. Each product is thoroughly investigated and its merits considered before it is permitted entry.

Semen from cattle, sheep and swine, from foot-and-mouth disease or rinderpest countries is prohibited.

It will be interesting for you to learn that since the end of World War II there has been a great movement of people between the United States and Europe, people who have been going back to the homeland or people who have been coming to visit relatives here, and it would seem for a while that most everyone in foreign countries thought that we here in America were hungry for meat and for that reason they brought great quantities of dried sausages, salamis, and other meat delicacies in their baggage, which Customs seized for us for disposal. We have in the past seized as much as 160,000 pounds of meat out of passenger baggage in one year and currently we are taking from five to 6,000 pounds a month. Customs inspection of passenger baggage, including searching for and removal of restricted meats, plants, seeds, and so forth, is made. These articles when found are seized and generally destroyed. Because of reduced funds in recent years, baggage inspections were seriously reduced. The ARS budget for fiscal year 1957 included a request for $856,000 to be used by Customs to restore previously established standards of baggage inspection at maritime ports and
airports. At the same time, $163,000 additional funds have been allotted to strengthen the technical quarantine service at ports of entry. Meats usually found in passenger baggage are potentially dangerous because most of these sausages are made from ground beef, pork, and veal—seasoned, dried, and not cooked. The danger lies in the fact that a lot of our people would disdain eating such a product and when the friends and neighbors have gone, they throw it in the garbage can and there is the grave danger of the herds of raw garbage feeders becoming infected.

With the introduction of the airplane into the handling of livestock movements, we are faced with graver problems than we ever have been in the past. Most shipments coming from Europe, Asia, and Africa would take from 15 to 40 days by boat, and, in some instances, we would have knowledge of disease prior to the arrival of the ship and could properly control the arrival of the animals. However, since July 1, 1955, we have received at the port of New York about 88 percent of the poultry importations by air and about 67 percent of the horse importations by air, and numbers of cattle and zoological animals, the flight time of which is about 12 to 14 hours. This, you will understand, adds to our responsibility as exposed animals might not have had an opportunity to develop symptoms of a disease prior to the landing but still would be infected and would constitute a danger to domestic livestock unless we had adequate quarantine facilities.

We are encountering considerable difficulty with the various research establishments that are using serum as a base for numbers of their experiments, which they receive from foreign countries, and, of course, these must be held up until we are satisfied that the serum used was not of ruminant or swine origin or has been heated to a temperature which was safe for introduction into this country.

You might be interested in knowing that there have been times at the port of New York when over a million wet salted cattle hides have been imported during a month's period, from infected countries, and these must be handled off the ship, across the pier to sealed cars, trucks and lighters and sent to tanning establishments that are operating under the supervision of this Branch for disinfection of such hides and skins.

Quarantine and regulatory personnel are kept informed of the presence and the distribution of animal and plant diseases in the various foreign countries. This information determines to some extent the decisions that are made for issuance of permits for importations of animals and plants.

Health certificates or inspection certificates are required for importations of animals, plants, seeds, bulbs, and so forth, brought into the country. Upon arrival of such shipments from foreign countries by boat or air, inspection is made to determine the presence or absence of diseases or pests. This service is conducted in cooperation with the Customs Service, the technical work being done by representatives of the plant and animal quarantine branches of ARS. Animals and poultry are placed in quarantine for a period of time sufficient to be assured that they are free of communicable animal diseases or exposure thereto.

I have a number of slides I should like to show you, thank you.
REPORT OF THE COMMITTEE ON EXOTIC DISEASES


The Committee has devoted its efforts to reviewing the activities and progress made by the State and Federal governments relative to prevention, diagnosis and control of foreign animal diseases. All states have indicated an interest and progress in this emergency program during the year. The progress within each state, of course, varies, some having developed and established organizations that could function promptly if and when an unusual or foreign disease is reported. Others still require much attention and work.

The Committee, however, does feel that there is an increased interest and awareness by the State and Federal regulatory services of the potential threat of foreign animal diseases to the livestock of this country. Increases in foreign trade and travel, especially by air, have brought us face to face with this peacetime danger. Within many states the subject of foreign animal diseases has been included at state and local veterinary meetings. Papers and articles have been published and distributed, and the various livestock owners and organizations made aware of this threat.

In addition to state and local meetings, the Agricultural Research Service, in cooperation with the State livestock sanitary officials, conducted a series of regional meetings during the spring of 1956. These meetings were held as indicated below:

- Phoenix, Arizona—March 15–17
- Stillwater, Oklahoma—March 19–21
- Bozeman, Montana—March 22–24
- Ames, Iowa—March 27–29
- Atlanta, Georgia—April 9–11
- Storrs, Connecticut—April 17–19

Invitations were extended to State and Federal livestock sanitary officials, practitioners (through the secretaries of all state veterinary medical associations), the schools of veterinary medicine, departments of veterinary science or pathology in the land-grant colleges, and certain public health officials in each state. Special emphasis was placed on having at the meeting (1) veterinarians who have been designated to be in charge of the program for the control of emergency outbreaks of diseases in each state, (2) diagnostic laboratory workers, (3) college clinicians, (4) extension veterinarians, and (5) others who may be called in for consultation with practitioners confronted with unusual disease occurrences. Through this series of meetings it was hoped to have the key people from each state thoroughly familiar with the foreign diseases that are considered most
likely to gain entrance into the United States, and to inform them as to what organization has been planned for coping with these emergency outbreaks of foreign diseases. About 1,000 veterinarians and veterinary students attended these six regional meetings.

Proceedings of these meetings have been prepared and represent summaries of most of the discussions and talks presented. The material is current and should be useful in providing information and promoting discussion on the subject of foreign animal diseases. Information includes precautions taken by the Federal government to help prevent the entrance of foreign diseases into the country, the cooperative State-Federal disease control programs, the State-Federal emergency disease control and eradication programs, and discussions of several serious foreign diseases, including their symptoms and pathology. The need for prompt reporting is also emphasized. It is felt that if the material in the proceedings is widely used, it will provide a better understanding of the problems associated with foreign disease prevention and control. It will also help to encourage the cooperation of all segments of the veterinary profession and livestock industry in the protection of livestock and poultry of this country from exotic-type diseases. These proceedings will be distributed to those who attended the regional meetings. Additional copies will be available for those who desire them. This information will be made available to the veterinary colleges for their use.

Several states have followed up these regional meetings with meetings of their own, encouraging their state officials to discuss the subject in their local areas. The potential threat of foreign diseases and the responsibility of the local livestock owner and veterinarian to promptly report unusual or foreign diseases to regulatory authorities has been emphasized. Members of the Agricultural Research Service have been invited to appear before state meetings to discuss the subject of foreign animal diseases.

The Agricultural Research Service, in cooperation with the Department of Defense and the Federal Civil Defense Administration, has continued to develop colored sound movies on foreign animal diseases. During the past year a colored sound movie on contagious bovine pleuropneumonia was secured from the Australian Government and sufficient copies made for distribution to all veterinary colleges for instructional purposes and to those states having large cattle populations. A movie entitled “Hog Cholera—African Swine Fever, A Comparison” which describes and shows the characteristic pathology of hog cholera and African swine fever has recently been cooperatively developed by the Agricultural Research Service, Department of Defense, and Federal Civil Defense Administration. This film, too, has been distributed to veterinary colleges and to the states. A colored sound movie is now being prepared by the Agricultural Research Service on the means by which foreign diseases might gain entrance. This movie should be completed by June 1957. All movies, with the exception of contagious bovine pleuropneumonia, are listed and described in the United States Livestock Sanitary Association’s handbook on foreign animal diseases.

In addition to the movies, Kodachrome slides of the gross and histopathology of some foreign diseases have been developed and are available for instructional and reference purposes. In some cases, tissue slides have been prepared. As
material becomes available, additional sets on other foreign diseases will be developed and made available.

The development of procedures for the diagnosis of certain serious foreign animal diseases has not been completed at this time. It is planned to finish this project during the coming year. The determination, acquisition, and distribution of selected antigens, sera and antisera, and other required biologics needed for the diagnosis of certain foreign diseases is being developed in connection with the preparation of the procedures.

Neutralizing antiserum for use in connection with differential diagnosis of fowl plague is being produced at the Plum Island Animal Disease Laboratory in preparation for distribution to certain designated poultry disease diagnostic laboratories. Antisera against three strains of virus are now being processed and will be distributed in the near future.

A symposium on the vesicular diseases of animals was held on Plum Island September 27 and 28. The eleven papers which were presented and discussed by scientists from the United States and foreign countries will be published by the Department.

The Committee recommends that the educational program on foreign animal diseases be continued and stimulated. The Committee believes that the veterinary schools should be encouraged to give regular courses on foreign diseases and that they should be encouraged to use the visual aids available.
SHEEP AND CATTLE SCABIES ERADICATION

J. L. HOURRIGAN*

I shall not attempt to review in detail the long history of scabies eradication in the United States, since this group is familiar with it and many of you have played an active part in the program. However, I would like to mention, very briefly, some points of interest in regard to the early history of the disease. The history of scabies is one of the most fascinating chapters in the annals of medicine. The disease has been known since earliest times, and scabies mites were once thought to be the smallest form of living matter. Scabies is referred to in the Old Testament, and the mites are mentioned in Arabian medicine. The work of Bonomo and Cestoni, in 1687, revealing the causative relationship of the scabies mite to the disease, was of epochal significance: It marked the establishment, for the first time in the history of medicine, of a definitely known agent causing a known disease in man. It constituted, long before the advent of the eras of mycology, bacteriology, and virology, the first concrete proof of the theory of specificity in the etiology of infectious diseases. It also constituted an important link in the chain of development of the microbial origin of infectious diseases. The theory and ultimate acceptance of the acarian origin of scabies dealt a powerful blow to the doctrine of humoralism which, as a pathologic concept, had dominated medicine from the days of Hippocrates well into the 19th century.

Just how prevalent scabies was in the distant past is difficult to say. Undoubtedly the disease was a great deal more common than at present. Historically and geographically, scabies has been coextensive with man and his animals. Wars, major disasters, and other factors causing overcrowding and mass movements of men and animals create conditions that enhance the spread of the disease. Modern transportation and marketing have made the problem of scabies eradication particularly difficult in the United States.

Scabies in one of the oldest diseases known. We have had a satisfactory treatment for more than half a century. Scabies has caused tremendous losses in this country—neither cattle nor sheep that have it can be profitably grown.

PSOROPTIC CATTLE SCABIES

Psoroptic cattle scabies became such a serious problem in the United States at the turn of the century that in 1903 a Federal declaration was placed in effect involving the country west of the Mississippi River. From 1904 to 1929 reports indicate that psoroptic cattle scabies appeared quite regularly in the majority of the western states and in several of the eastern states. A large-scale eradication program was organized. From 1930 to 1947 the eradication program, based on the principles of quarantine, inspection, and dipping, enjoyed measur-

*Dr. J. L. Hourrigan, Chief, Special Diseases Eradication Section, Animal Disease Eradication Branch, Agricultural Research Service, U.S. Department of Agriculture, Washington, D. C.
able success and the disease situation improved. From 1948 to 1953 this country was relatively free of the disease.

In January 1954 psoroptic scabies was diagnosed in cattle shipped into Arizona from Colorado. During the remainder of fiscal year 1954 the disease also appeared in California, Colorado, Missouri, Oklahoma, Texas, and Wisconsin. The movements of cattle from infected and exposed herds were carefully traced and all known infection was eradicated.

The first report of psoroptic scabies in fiscal year 1955 was made in November 1954 from the Chicago Union Stockyards in reference to a shipment of cattle from Denver, Colorado. Further investigation disclosed that the diseased animals had been shipped from a ranch in Crowley County, Colorado, the same area in which scabies had been found the previous winter. Additional inspections in Colorado disclosed evidence of the disease in a total of 16 herds in six counties. The State of Colorado placed a quarantine on Kiowa, Costilla, Prowers, Baca, Bent, Otero, and Crowley counties and on parts of Pueblo, Huerfano and Las Animas counties. An all-out eradication program, including compulsory treatment of all cattle regardless of whether they were infected or known to be exposed, was begun on farms and ranches and at community sales within the quarantined area. Cattle moving from the quarantined area into other parts of Colorado or interstate were also treated. Since there is a substantial interchange of cattle between southeastern Colorado and adjacent areas in Kansas and Texas, it is not surprising that infected herds were also found in these States. In addition, infected herds were found in Illinois, Nebraska, and Kentucky.

During fiscal year 1956, psoroptic cattle scabies was diagnosed in Colorado, Iowa, Kansas, New Mexico, and Texas. The last known infection in Colorado was found on October 31 and November 1, 1955, in Crowley County, involving one previously infected herd and two herds on an adjoining premises. Fortunately, all cattle sold from the infected herds had been dipped at the time of sale. Psoroptic cattle scabies was diagnosed in one herd each in the States of Kansas, New Mexico, and Texas, and in cattle shipped from an Iowa farm to Sioux City stockyards. In both the Kansas and Texas outbreaks, cattle belonging to the same owners had been found to be infected during the winter of 1954–55. All known infected herds have been treated under supervision, and every effort has been made to trace movements of cattle from these herds and to establish the origin of infection in each case.

CHORIOPTIC AND SARCOPTIC SCABIES

The incidence of chorioptic scabies in cattle apparently has been increasing for several years, particularly in the midwestern states and the northeastern states. The increase of chorioptic scabies has made this disease an important economic factor in successful livestock farming and dairying.

During fiscal year 1956, chorioptic scabies was diagnosed in cattle at the International Livestock Exposition in Chicago, Illinois, the American Royal in Kansas City, Missouri, the Grand National Livestock Exposition at the Cow Palace in San Francisco, California, and the National Western Livestock Show in Denver, Colorado. Chorioptic scabies was reported also in California, Illinois, Indiana, Iowa, Massachusetts, Michigan, New York, Ohio, Oklahoma, Washington,
and Wisconsin. The disease is probably more widespread than the reports would indicate.

Sarcoptic cattle scabies has been found on both farm and range cattle in the United States over a period of many years. It has been particularly serious in dairy herds of the Midwest and eastern states, being generally spread by the sale and exchange of cattle. Sarcoptic scabies was diagnosed in fiscal year 1956 at the Chicago Exposition and at the Denver show. In most cases, the infected animals had previously appeared in other shows. Breeding bulls of the beef breeds have often been responsible for spreading sarcoptic scabies to range areas. The disease is much more common in swine than in cattle. During fiscal year 1956, sarcoptic cattle scabies was reported also in North Dakota and Wisconsin.

**Psoroptic Sheep Scabies**

Psoroptic sheep scabies has probably been present in the United States since the first sheep were introduced into this country, and has been very widespread from time to time. Efforts to eradicate the disease were begun more than 50 years ago. A Department of Agriculture bulletin on sheep scabies, published in 1898, contained excellent illustrations of scabby sheep, the causative mites, and equipment needed for treating ovines by dipping. In 1904 a Federal quarantine was placed upon the western half of the United States. The Bureau of Animal Industry report for 1910 states that “previous to the establishment of the Federal quarantine on scab there was almost no place in the United States that could claim more than a temporary freedom from the disease.” An active eradication program was developed and gradual progress was made in the western range areas. The Federal quarantine was removed from counties and States as the disease was eradicated. However, reinestation of sheep in range areas was not uncommon and it was not until some 20 years ago that the eradication program in the West neared completion. As the disease became less common in these areas more attention was directed toward its eradication in the Midwest and East. An active eradication program was instigated in Louisiana and in Mississippi, particularly in certain parishes and counties that had been under Federal quarantine since 1918 and 1927. This all-out drive has been quite successful and has eliminated one of the important reservoirs of infection.

Unfortunately the general outlook in other States, particularly those of the midwestern farm areas, is not nearly so good. The disease probably has been present in the majority of the problem States almost continuously since before 1900. Active eradication programs have been followed from time to time in a number of these States but, for the most part, have afforded only temporary relief. In fact, official reports indicate that the incidence of sheep scabies is gradually increasing. The following figures report the numbers of States, counties, and flocks affected during fiscal years 1954, 1955, and 1956:

<table>
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<th>Flocks</th>
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<td>21</td>
<td>183</td>
<td>391</td>
</tr>
<tr>
<td>1955</td>
<td>24</td>
<td>219</td>
<td>442</td>
</tr>
<tr>
<td>1956</td>
<td>25</td>
<td>267</td>
<td>607</td>
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A comparison of the annual report for 1956 with that for 1955 indicates there was an increase of 37 percent in the number of infected flocks reported and an increase of 22 percent in the number of counties involved. The report of 607 infected flocks in 267 different counties (an average of approximately 2.3 infected flocks per county) suggests that the disease is rather widespread in certain areas. In addition to the information included in this report, psoroptic sheep scabies was diagnosed in 110 consignments of sheep received at public stockyards under supervision of the Animal Disease Eradication Branch.

On March 1, 1955, the Department, at the request of the industry, called a meeting in Chicago to review the problem of sheep scabies. The following points received particular consideration:

1. **Means of Spread**
   
   (a) Through purchases from public stockyards, auction or sales rings, and other concentration points.
   
   (b) Through extensive movements by farmers, dealers, truckers, and by rapid modern transportation.
   
   (c) Through lax dipping requirements during winter months.
   
   (d) Through purchases of infected breeding animals, particularly rams.
   
   (e) Through difficulty in detecting scabies in its early stages by routine inspections at concentration points.

2. **Miscellaneous**
   
   (a) Diversion of shipments enabling sheep to enter a State unknown to sanitary officials and without meeting sanitary requirements.
   
   (b) Diversion of undipped sheep billed for immediate slaughter but re-entering commercial channels.
   
   (c) Reinfection of clean areas from other areas and from other States in which reservoirs of infection continue to exist.
   
   (d) Shortage of veterinarians and other trained personnel who are experienced in the diagnosis and eradication of scabies.

The Agricultural Research Service was asked to develop a tentative Sheep Scabies Eradication Program to be sent to all State livestock sanitary officials for review and comment. A proposed Industry-State-Federal Sheep Scabies Eradication Program was developed and sent to State livestock sanitary officials on April 27, 1955. I am sure you are all familiar with the proposed program which included (1) education, (2) training of personnel, (3) a suggested program for States where scabies has not existed within the past 12 months, and (4) a suggested program for States where scabies has existed within the past 12 months. You will recall that the latter included (a) an active inspection program to locate infected flocks and reservoirs of infection; (b) State quarantine of all infected and exposed sheep until properly treated under supervision, and quarantines covering areas where the disease is widespread; (c) Federal quarantine when needed to protect other States; (d) dipping of all sheep returning to farms from shows, sales rings, and stockyards; (e) encouraging the dipping of all sheep on change of ownership; (f) dipping all sheep before they move into other flocks or enter shows; and (g) locating the source of scabies in infected flocks and tracing all movements from such
flocks in order to uncover and eliminate the disease at its source and in subsequent shipments.

The proposed program received wholehearted support from State livestock sanitary officials and members of the industry.

In regard to education and training of personnel, I am pleased to report that we have made good progress. On April 10, 1956, we distributed a kit of 45 color slides showing scabies mites, cattle and sheep affected with scabies, and methods of diagnosis and eradication, to each Field Inspector in Charge to train employees in scabies eradication and to show to interested livestock groups. We have also just completed a color-sound film on sheep and cattle scabies. This film will be ready for distribution in the near future and should prove quite helpful as an educational tool. As an additional important aid in the training of personnel, arrangements have been made with the Animal Disease and Parasite Research Branch to use their facilities at Albuquerque, New Mexico, to give on-the-spot training to employees. To date, 52 Federal and State employees have taken advantage of this opportunity to further their practical knowledge in regard to the diagnosis, general understanding, and eradication of sheep and cattle scabies.

Experience has shown that both Federal and State authority is needed for satisfactory control, whether the proposed movement of sheep is intended to be interstate or intrastate. In some cases, States alone have been unable to control the spread of sheep scabies. On the other hand, Federal quarantine power is incomplete within the State. Basically, the problem of scabies eradication falls upon the States themselves. Federal funds are not sufficient to assume a large share of the cost of an all-out sheep scabies eradication program. The States concerned will be expected to provide the bulk of the monies necessary.

In scabies we have a disease that is not overly difficult to eradicate. We have excellent acaracides that can do the job. We have established eradication principles and procedures that have proved again and again to be effective. There is a need for educational work and trained personnel. Particular attention should be paid to dissemination of the disease through concentration points. Systematic inspection is required to detect reservoirs of infection. Quarantine must be invoked when needed. Dipping must continue during the winter months. Complete eradication is possible. But it depends upon the full cooperation of all livestock disease control agencies and the livestock producers of the Nation.
SHEEP SCABIES CONTROL IN OHIO

JAMES E. DORAN, DVM

Division of Animal Industry, Ohio Department of Agriculture

Ohio has the greatest concentration of sheep of any state in our country. Nearly two million sheep are kept by some sixty thousand sheepmen in small farm flocks, which average thirty to thirty-five sheep. Last year one hundred fifty-two of these flocks were affected with common body scab for an infection rate of one fourth of one percent. These one hundred fifty-two flocks were quarantined; they were reported to the Agricultural Research Service; they were dip treated under state or federal supervision, and they were released from quarantine after subsequent inspections revealed that they were free from scabies. Three of these flocks had to be retreated to effect a cure. Even though this infection rate is lower than that of certain other diseases of our livestock, we know that it greatly hinders our purebred industry by interstate restrictions; it hinders our livestock marketing people; and it stands as a threat to our farm sheepmen. We recognize sheep scabies as a serious menace to our total sheep industry. We are obligated to promote and protect that industry.

Theoretically, scabies could be controlled in a state by law, rule, or regulation. We could enact such interstate and intrastate regulations as would appear to preclude any dissemination of scabies through the movement of infected or exposed sheep. We all recognize that regulations, alone, will not do the job. We only, as the saying goes, “strain a gnat and swallow a camel”. In addition, it puts us against the sheep industry which is struggling to hold its ground in the total livestock industry. It needs our assistance as surely as we need its cooperation in our disease problems.

In Ohio, we depend on the importation of western ewes and lambs to maintain our brood flocks and feedlots. We waive the dipping on these sheep and allow them to be admitted by permit or inspection certificate. This has proved sound from a disease control standpoint and we have gained additional industry support. We do not dip sheep at our local markets for intrastate movement. Our records show that scabies was controlled and eradicated following an intensified program of inspection by market operators, their veterinary inspectors, state and federal veterinarians and lay inspectors, in conjunction with an active dip treating program in the field under state and federal supervision. This program accomplished what dipping at our local markets did not do. We feel that it was successful because it placed control and eradication of disease in the hands of professional and trained personnel, working under a unified program from a state-wide level, in cooperation with the segments of the industry.

Most of our twenty-three state and federal area veterinarians have had field experience with sheep scab. They can carry out the investigations, issue quarantines, supervise the dip treating and make the subsequent inspections for the release of quarantines. In addition, we have five lay market inspectors who have been trained in scabies inspection and treatment. They carry out much of the
tracing from affected flocks and assist in the supervision of treating. We utilize
the services and equipment of our commercial sheep dippers for field treatment
unless distance makes it impractical. We have three dip tanks and drain racks, car-
ried on small, two-wheeled car trailers which we can furnish in those areas where
the services of a commercial diper is not readily available and for use in winter
months when the dip treating is done inside.

We are presenting the problem of sheep scab to all segments of the industry.
In veterinary meetings, in veterinary publications and in our veterinary college
we are emphasizing veterinary responsibility and cooperation. We are working
with market operators, individually and through their marketing organizations,
with The Ohio Sheep Improvement Association, The Agricultural Extension Serv-
ice, The Ohio Sheep Shearer’s Association, and various county sheep organizations
and vocational agricultural groups. In addition, the scabies situation and our pro-
gram has been given state-wide coverage through farm magazines, from radio and
television programs and featured press articles by farm editors. We support these
organizations with our services and give their programs a boost whenever possible.
We feel that they believe in what we are trying to do for the sheep industry and
we are certain that we need their help to get the job done.

The continued support of our program depends on its effectiveness in the field
from a farm level. Like other diseases, no uniform regimen of control and treat-
ment will work in every case. The condition of the sheep, stage of the disease,
weather conditions, shearing dates, lambing dates, and marketing dates influence
the procedure. We put the flock under quarantine and set up the earliest date for
the dip treating which best fits the total situation. Inopportune dipping may give
curative results of scab in a flock but its side effects do not promote the program.
Benzene hexachloride or Lindane wettable powders in a suspension of six hun-
dreths of one percent gamma isomer or Toxaphene in a solution of one half of one
percent are used exclusively for dip tank solutions. We feel that they are about
equal from both a curative and toxicity standpoint. Properly used, they will effect
a cure in all but a few severely advanced cases, with one treatment. Fat lambs may
be removed from quarantine thirty days after dipping, on permit for direct
slaughter. The remainder of the flock is released ninety days after dipping, pro-
vided our inspection reveals freedom from scabies. The quarantining, dip treat-
ing supervision, cleaning and disinfection, inspections and quarantine releases is
carried out entirely by state and federal veterinarians and trained lay inspectors.

Briefly, our situation is this. We recognize sheep scabies as a serious menace to
our sheep industry which we are obligated to promote and protect. We have a
program which we feel best fits our problem and is commensurate with funds and
personnel. We are getting it to the sheep industry for its support, and we are back-
ing it in the field with the best we can give it. We are controlling sheep scab in
Ohio with this program. We may accomplish eradication with this program again
as we did in 1946, 1947 and 1948. Doctor James L. Hourrigan’s recent review of the
history and incidence of sheep scabies in our country illustrates that eradication of
this disease on a state basis can be, at best, only a temporary accomplishment for
many states. We believe that our program in Ohio contains some of the basic
principles of a sound national eradication program in which lies the real answer
to the sheep scabies problem of every state.
REPORT OF THE COMMITTEE ON PARASITIC DISEASES


Since there is an increased prevalence of scabies in sheep in certain areas and also an increase in various types of mange in cattle, the report of the Committee on Parasitic Diseases is on the subject of eradication of the disease from this country. In cattle, we are seeing more Demodectic mange, and other types of mange in cattle are becoming a problem. Scabies in sheep is again becoming a serious threat to our sheep industry.

Mange in animals has been known and reported in the earliest historical records. At times it has been a great scourge, at other times it has been brought under partial control, but it still exists in some form to plague the various animal industries and cause enormous losses. Mange or scabies in cattle and sheep is still a common disease occurring in every part of the world. In the United States, the disease in the past has been widespread, but in many sections, it has been brought under complete control by eradication, although in some areas, especially the midwest farm areas, the spread of scabies in sheep has been increasing.

The mites causing scabies or mange in sheep belong to five genera: Psoroptes, Chorioptes, Sarcoptes, Demodex, and in 1951, a new report in the United States on Psorergates. These mites causing scabies in various forms are probably the most destructive and persistent of sheep diseases.

The mite usually causing the greatest damage is the Psoroptes or common scab mite. The others are of less economical importance or occurrence but are still a national problem to be considered. The Chorioptic or symbiotic mite causing foot scab or leg mange is very common in some sections, but does not cause as great economical losses as the common scab mites. The mites and lesions are often only on the lower parts of the legs and around the feet, but in some severe cases the mites may spread to other parts of the body such as the thigh, udder, abdomen, and tail head. Sarcoptes scabiei causing head scabies is an European disease not commonly found in this country. Demodectic mange while thought to be rare in this country is probably fairly common as reports from New York have shown the mites are commonly found in the eyelids of sheep. The Psorergates mite which usually affects fine woolled sheep was reported in Ohio sheep during 1951. The Psorergates mites (Psorergates ovis) does not produce the severe symptoms as some of the other mites as the disease spreads slowly and may be in the flock for a long time before it is diagnosed. There is a mild irritation, and pulling of the wool from the infected areas, giving a ragged appearance to the fleece.

The prevalence of each species in these animals varies in its occurrence in areas of the United States.

Psoroptic scabies or mange in sheep at one time was present in almost every state with vast economical losses, but after the Federal Quarantine was placed on the movement of sheep, the condition was brought under partial control and the
great western part of the United States was completely free of the scourge. The Bureau of Animal Industry annual report for 1910 states that “Previous to the establishment of the Federal Quarantine on scab, there was almost no place in the United States that could claim more than temporary freedom from the disease.” At the present time, psoroptic scabies is present chiefly in the midwestern, and some southern and eastern states.

In the annual report of Cooperative State-Federal Sheep and Cattle Scabies Eradication, July 1, 1955, to June 30, 1956, Dr. J. L. Hourrigan, Chief, Special Diseases Eradication Section, United States Department of Agriculture, comments on Psoroptic Sheep Scabies as follows:

“A comparison of the annual report of sheep scabies for 1956 with that for 1955 indicates there was an increase of approximately 37 percent in the number of infected flocks reported and an increase of approximately 22 percent in the number of counties involved. The report of 607 infected flocks in 267 different counties (an average of approximately 2.3 infected flocks per county) suggests the disease is rather widespread in certain areas.

In addition to the information included in this report, psoroptic sheep scabies was diagnosed and mites demonstrated in 110 consignments of sheep received at public stockyards under Branch supervision.”

Chorioptic mange commonly called foot mange is also common in sheep in some sections. It does not cause the great economical losses as the Psoroptes, but in many cases much discomfort is seen. Sarcoptic and Demodectic mange while reported in sheep probably does not cause great economical losses in this country.

The mites found on cattle belong to the genera: Chorioptes, Psoroptes, Sarcoptes, and Demodex. The common scab mite, Psoroptes, at one time, was the most prevalent in the United States, but the incidence has been changing and now Psoroptic mange is relatively rare with only a few cases being reported in this country during the past year. Sarcoptic and choriopic scab is now replacing the common scab mite in prevalence.

Again the annual report by Dr. J. L. Hourrigan states: “During Fiscal Year 1956, psoroptic cattle scabies was diagnosed in Colorado, Iowa, Kansas, New Mexico, and Texas. The last known infection in Colorado was found on October 31, and November 1, 1955, in Crowley County, involving one previously infected herd and two herds on an adjoining premise. Fortunately, all cattle sold from the infected herds had been dipped at the time of sale. Psoroptic scabies was diagnosed in one herd each in the State of Kansas, New Mexico, and Texas, and in cattle shipped from an Iowa farm to the Sioux City, Iowa, Stockyards. In both the Kansas outbreak and the Texas outbreak, cattle belonging to the same owners had been found to be infected during the winter of 1954–1955. All known infected herds have been treated under supervision, and every effort has been made to trace movements of cattle from these herds and to establish the origin of infection in each case.”

Chorioptic mange at one time, considered rare in the United States, has become quite prevalent in many parts. The mites are commonly found along the inside of the legs but in many cases they can be found in other parts of the body. They cause considerable irritation with the animal biting and scratching. In the Annual report
of Cooperative State-Federal Sheep and Cattle Scabies Eradication, the following is reported:

"During Fiscal Year 1956, chorioptic scabies was diagnosed in cattle at the International Livestock Exposition in Chicago, Illinois, the American Royal in Kansas City, Missouri, the Grand National Livestock Exposition at the Cow Palace in San Francisco, California, and the National Western Livestock Show in Denver, Colorado. Sarcoptic scabies was diagnosed at the Chicago Exposition and at the Denver Show. In most cases, the infected animals had previously appeared in other shows. Chorioptic cattle scabies was also reported in California, Illinois, Indiana, Iowa, Massachusetts, Michigan, New York, Ohio, Oklahoma, Washington, and Wisconsin."

Sarcoptic mange in cattle in also increasing in prevalence and becoming widespread. In his annual report, Dr. J. L. Hourrigan states: "Sarcoptic cattle scabies was also reported in North Dakota and Wisconsin. This disease was reported in swine in New Mexico and Tennessee and is thought to be more prevalent in this species than generally reported."

Demodectic or follicular mange in cattle is far more prevalent in the United States than is thought. Surveys have shown it to be a very common mange in many dairy herds, breeding herds, and show animals at State and County Fairs. The disease is often not diagnosed because of the general lack of knowledge of its prevalence and identification. The lesions are found commonly in the region of the neck and shoulders. As the disease progresses, small papules develop which later form abscesses filled with numerous mites. These abscesses will vary in size from one-quarter to almost an inch in diameter.

With the modern insecticides and their great efficiency in destroying mites in animals, a new era has come into play in the control of mange. Since it is possible to control scabies in sheep and cattle, there seems to be no reason for the continual danger of the spread of scabies again to the Western Range states and other areas. With the recent upsurge in the prevalence of the disease in sheep in the midwestern states, it is possible for this disease to become firmly settled again before it is stopped. Half-hearted methods are not enough to control the mange. It has been found that a persistent campaign is needed to completely eradicate the disease and even when the condition seems to be licked, it is most important to keep checking and controlling isolated cases that appear. In many cases of mange, the disease will appear controlled after one or two treatments but unless follow-up methods are employed, the condition will again become prevalent.

What is needed, in a complete eradication of the disease, is a campaign to locate and treat all cases of mange and then keep checking herds to control all outbreaks. The state reporting-service should be relied upon to report such cases to the authorities so that immediate steps can be taken to control the outbreak before it can spread.

There seems to be no need for this disease to cause such ravaging economic results to both the sheep and cattle industry when there are effective measures at hand to completely eradicate it.

The report of this committee is not to advance any theories or methods to combat or eradicate the disease, but rather to reiterate and bring to this body the fact
that scabies can be eradicated from this country and that measures and methods now in use should be used to their fullest in combating this disease. For many years, programs to eradicate scabies have been inaugurated but usually they fall short of complete eradication.

The committee should also like to point out that consideration should be given to the control of scabies-infected herds or individual animals in exhibitions and at fairs. It is not uncommon to find animals with various types of mange being exhibited at fairs and being transported between various fairs and shows in one state and then being transported to fairs and shows in another state. One type of mange (Demodectic) in cattle can be found quite commonly and nothing seems to be done about the shipping of these cattle interstate. As has been shown by the various reports, chorioptic and sarcoptic mange has been found at various expositions and fairs during the past year. This in itself serves to spread the disease rapidly over wide areas.

The committee would also like to point out that the recommendations resulting from the Sheep Scabies Meeting in Chicago, March 1, 1956, has already begun to “bear fruit”. The recommendations for an educational and training program has been followed by the issuance from the Animal Disease Eradication Branch, Agricultural Research Service, United States Department of Agriculture, of a series of 45 colored scabies slides of cattle and sheep showing the lesions, the various mites involved, the methods of diagnosis, and manner of eradication. A complete narration series correlated for use with slides was sent with the slides. The slides and narration series were sent to each Animal Disease Eradication Branch Field Inspector-in-Charge for general use within the state concerned and to each Veterinary College. These slides were to be used to train persons in the various phases of scabies eradication and also be available for showing to interested livestock groups. These are very excellent slides, the narration is well-prepared, interesting, and educational; the whole series can be used to great advantage to aid in the eradication of scabies. The slides received at the Veterinary Colleges are excellent for teaching the fundamentals of scabies eradication to veterinary students and could be used to further advantages by any group that would be interested in the eradication of scabies. The other points in the tentative Sheep Scabies Eradication Program of the Animal Disease Eradication Service should be closely followed to completely eradicate scabies from this country.
STATE WIDE TESTING FOR PPLO INFECTION OF POULTRY*

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Department of Poultry Husbandry, University of New Hampshire,
Durham, New Hampshire

While the pleuropneumonia-like organism (PPLO) may be isolated from many species of domestic animals, it was in 1943 that Delaplane (1) first isolated these organisms and pointed out their role in chronic respiratory disease of birds. Some nine years later the problem had assumed such magnitude in the poultry industry that a conference was called in Washington to determine what might be done to eventually reach a means of control of the disease. According to the report submitted last year by Dr. O. L. Osteen to this Association, considerable advance has been made in the laboratory during the three year interval. This is particularly true in regard to isolation of PPLO, serological tests, and the detection of spread through the hatching egg.

However, very little has been accomplished in the actual control of PPLO infection under field conditions. There are two major difficulties which probably have created this condition.

1. The technical difficulties found in working with the PPLO.

2. The inability of PPLO cultures alone to produce mortality and severe morbidity found in the air sac disease of chickens.

Upon considering these two factors, particularly the latter, considerable caution has been exercised by investigators in regard to field control of PPLO infection. Since the publications by VanRoekel (2), Fahey (3), et al., concerning hatching egg transmission as one of the sources in the spread of PPLO, considerable interest has been stimulated in the hatching industry. As New Hampshire is essentially a hatching egg state, keen interest was developed with respect to breeders. Were they positive or negative to the PPLO serological test and what might be expected of their progeny?

From the outset an approach similar to the pullorum disease eradication program might appear to serve as a good hypothesis. However, it is easily observed that such a direct approach might be quite impracticable. You may recall the problems in the early 1930's when a 30 percent reactor rate to S. pullorum was not unusual, and when relatively inexpensive strains of birds were involved. This is in contrast to the present high reactor rate to PPLO in the small expensive genetic nuclei which supply the breeding stock of our industry. However, the industry as a whole realizes that research must be intensified in this area to solve the problem of CRD.

The most interested group in New Hampshire were those geneticists having the sources of the expensive blood lines. By working with these poultrymen, to the

* Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 200.
necessary exclusion of all others, our numbers of breeding flocks under field experimentation are small. 

The project actually began in early 1955. At that time five leading breeders in New Hampshire who were submitting chicks to the New Hampshire Broiler Test allowed us to random sample their breeders for PPLO. The results of these tests were quite remarkable in that the reactor rate was well above the 80 percent level and even as high as 100 percent in some of the flocks.

Upon accumulation of this data it was decided to sample test their progeny then currently available in the New Hampshire Broiler Test. In addition, known negative PPLO control chicks were raised under somewhat similar, but isolated conditions.

All chicks were vaccinated for Newcastle disease and infectious bronchitis by the dust method during the first week of life and revaccinated at three to four weeks. At eight weeks these chicks came down with a respiratory disease which developed into the air sac syndrome. Some mortality occurred. The clinical symptoms at this time were found to be incited by a virus which materially assisted in the spread of the hatching egg transmitted PPLO.

The interpretation of Table I is of interest. One of the most unexpected observations was the appearance of PPLO negative chicks from all breeders at five weeks of age. This is extremely interesting when it is noted that all chicks except the negative controls came from pens having 80 to 100 percent reactors from the random sampling techniques.

Of particular interest were the progeny from farm No. 4 which showed a reac-

### Table I

<table>
<thead>
<tr>
<th>AGGLUTINATION TEST - PPLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>% POSITIVE</td>
</tr>
<tr>
<td>BREEDERS and OFFSPRING</td>
</tr>
</tbody>
</table>

![Graph showing percentage positive for breeders and offspring over time.](image)

**KEY**:
- Farm 1
- Farm 2
- Farm 3
- Farm 4
- Farm 5
- Vacc. Control
tion rate of 45 percent at 8 weeks. At 12 weeks a 100 percent reaction rate was attained in this group while all other groups exhibited a lower percent positive. At 20 weeks all but one group were 100 percent positive; this group being above the 90 percent level. This undulating pattern of serological tests has been reported by other workers and appears quite typical of New Hampshire flock conditions.

The publication of Fahey and Crawley (4) on a proposed plan for the control of chronic respiratory disease of chickens by the use of antibiotics was of interest at this time. Their experiments were later followed by somewhat similar trials in California (5) and New Hampshire (6).

In regard to the New Hampshire trials, according to Weston (6) et al., it became evident that the injection of dihydrostreptomycin did not completely prevent hatching egg transmission of PPLO. The number of infected chicks hatched was small, and according to serological tests at 10 weeks varied from one in 300 on one farm to one in 1400 on another farm.

Eventually approximately 18,500 breeders in New Hampshire were involved in a project with eight hatcheries on a state-wide basis. For the purpose of this report however, only four cooperators have proceeded far enough to produce sufficient information on the isolation of brooding, rearing, and housing the experimental offspring.

The methods of obtaining PPLO negative chicks by the use of dihydrostreptomycin injected into positive breeders has been described by Weston, Strout et al. (6). The hatching eggs were saved nine days following injection and thereafter as long as the injections were continued (every two weeks in some instances). Each farm was requested to isolate the chicks in the most suitable manner within the limits of their overall plan for genetic research. As a result four general methods of isolation were used. Some have apparently proved of value while others have failed.

In all instances the eggs were washed with a commercial egg sanitizing solution, and the chicks were hatched in isolation.

Three commercial experimental antigens of two types were used throughout the report. The differences between the way these antigens react with immune sera appears to be great.

However, these variabilities appear to be no greater than differences found among batches of antigen from the same source. Positive sera has been used as a means of standardization but considerably more work is needed in this area. The production of antigen in sufficient volume appears at times to be a problem; especially when testing in volume is conducted.

EXPERIMENT I

In this experiment 2,000 breeders were injected. Each hatch was divided into five groups of approximately 2,500 chicks each. Each group was placed on a separate farm. All chicks were vaccinated with combination Newcastle disease and infectious bronchitis vaccine at four days and again at three weeks. Six hatches are reported in this paper. All birds were first tested for PPLO at 10 weeks. In one hatch all five farms remained negative. Two hatches produced four negative farms, and three hatches produced three negative farms.

Following testing the negative units were removed to a range divided into groups
by a fence. These groups were retested at 17 weeks when they were housed with two units reacting, and then again at 24 weeks when the pullorum samples were taken. This operation started with 42,522 chicks and has, to date, following testing and culling, approximately 10,478 negative PPLO breeding stock 24 weeks of age or older.

**EXPERIMENT II**

In the case of the second operation only one hatch was observed. The hatch was split into groups, all housed in one building. Each group was brooded in a separate pen. The results of this experiment may be seen in Table II.

The hatching egg transmission rate of PPLO was apparently very high. Cross infection became a problem, particularly when vaccination with combination Newcastle disease and infectious bronchitis vaccine was being carried out at one week of age. In fact the hatching egg transmission rate of PPLO was sufficiently great that the air sac syndrome was produced when the chicks were vaccinated. This occurred in pen B of one wing of the brooder house and a mortality of well over 3.5 percent resulted. This group in particular never overcome their respiratory symptoms and were discarded after the sample test.

Pen A of 925 birds exhibited no spread of infection when one positive was detected at 10 weeks. This pen was nearest the main entrance and was reached before
TABLE II

Results of Serological Tests for PPLO—Farm 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Number Birds Tested</th>
<th>Age 1st Test Weeks</th>
<th>Number Reactors</th>
<th>Age 2nd Test Weeks</th>
<th>Number Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>925</td>
<td>7</td>
<td>1</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>933</td>
<td>5</td>
<td>50</td>
<td>No test</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>982</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>D</td>
<td>979</td>
<td>7</td>
<td>0</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

pens C and D. The effect of the management is clearly shown here in that 17 reactors appeared in pen C and four reactors in pen D at the 10 week test. Later tests at seventeen weeks uncovered additional positives and respiratory symptoms were present.

It is evident that the hatching egg transmission rate continued to be an important factor in this flock even when the breeders were injected at the rate of 200 mg. per bird every two weeks. It is interesting to note that this is the only cooperator in the experiment who continuously used dihydrostreptomycin for increasing the quality of his chicks. The organism was isolated and did show some resistance to streptomycin in embryos. However, the strain is now lost through contamination with another strain of PPLO which apparently was harbored in the eggs before they were inoculated.

EXPERIMENT III

The breeders used in this experiment were similar in history to the two previous trials. However, no serological test on the parents was run prior to streptomycin injections. Some 2,500 breeders were involved. Following injections of dihydrostreptomycin, ten hatchings were made, and each hatch was placed on a separate farm.

Respiratory symptoms were observed on one of these farms at eight weeks of age, so the entire farm was discarded without the use of any serological tests.

On a second farm respiratory symptoms appeared at eight weeks in one pen and at 11 weeks in four more pens. The remaining pens were tested at 13 weeks with no reactors. At 19 weeks these pens showed mild respiratory symptoms, so a partial blood test was conducted. Reactors were found, so the farm was eliminated.

A third farm was all negative at the 12 week test. At 18 weeks of age, mild respiratory symptoms appeared in two pens on the bottom floor, so they were discarded. All other pens were tested an additional two times, both of which remained negative.

All the other seven farms were completely negative to two tests, one at 12 weeks and the second at 18 weeks. No symptoms have appeared in any of these units. As in the first experiment the value of isolation by small farm units may be seen.

In the case of the second farm, experiment 3, attempts were made to withdraw infected pens and keep the remaining negative birds. This proved unsuccessful. In the case of the third farm early removal of pens with respiratory symptoms left the remaining pens negative, and on two successive tests no spread of respiratory symptoms occurred in their groups.
EXPERIMENT IV

One thousand breeders were used, which from previous serological tests indicated a reactor rate of 100 percent. Dihydrostreptomycin injections were carried out and five hatches made of about 2000 chicks each. The hatches were all housed in one building, one hatch per pen. The first tests were carried out on these offspring at approximately 12 weeks, and the reactors removed. Positives appeared in all hatches except the first two.

At 17 weeks four more positive birds were removed from the first hatch. Additional testing was carried out at the time of the pullorum test with no reactors appearing. Shortly after the second test was completed, mild respiratory symptoms appeared throughout the entire flock, which lasted about two days. Tests six weeks later were all negative to PPLO.

SUMMARY

1. The availability and standardization of the antigen are of the utmost importance.
2. While dihydrostreptomycin suppresses hatching egg transmission of PPLO in some hatches, many of these hatches will have a small number of chicks infected.
3. There appears to be some advantage in the dividing of large hatches and isolating these groups until it is found whether or not they are infected with PPLO. Where this was not practiced considerable spread of PPLO occurred under virus infection.
4. Under commercial conditions it is possible to raise birds beyond 24 weeks which are negative and apparently have no infection with PPLO. These birds are progeny of breeders having a reaction rate to PPLO of over 80 percent upon random sampling serologically. It is very evident that the work is definitely in the field of research and it will be some time before we can approach the problem from the degree of accuracy that is found in pullorum testing.

REFERENCES

IMMUNOLOGIC DIFFERENCES IN STRAINS OF INFECTIOUS BRONCHITIS VIRUS*  

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Infectious bronchitis (IB) is the most wide-spread and diffusible respiratory disease of chickens. Economic losses are due to mortality in baby chicks, insufficient gains during the growing period, and prolonged decrease of egg production and egg quality in layers. The disease was first recognized as an entity by Schalk and Hawn (1), its viral etiology shown by Bushnell and Brandly (2), cultivability of the virus in chicken embryos by Beaudette and Hudson (3), and existence of parental immunity in chicks by Jungherr and Terrell (4). A general account of the disease is given by Hofstad (5).  

In the absence of effective remedies protection against this disease has been obtained by accidental natural exposure, by intentional exposure to fully virulent virus during the growing period, and by individual or mass vaccination with mild or attenuated, egg-propagated virus strains anytime during the nonlaying period. In this connection serologic and immunologic similarity of different IB strains has been assumed.  

In checking the results of immunization by challenge, Hofstad (6) made preliminary observations on antigenic differences in IB virus strains, and believed that this phenomenon should be considered in vaccine evaluation.  

The present report is concerned with studies which suggest plurality in infectious bronchitis virus.  

MATERIALS  

Four strains of IB virus were used in this study, with the following history, credit to contributors, and record numbers:  

Strains  

No. 66579, a completely egg-adapted, non-immunogenic strain, received as allantoic fluid from Dr. C. H. Cunningham, Michigan State University, East Lansing, Michigan, on May 21, 1947, originally isolated by Beaudette and Hudson (3), and now in its 238th embryo passage, usual titer in eggs $10^7$.  

No. 82828, isolated by Dr. Henry Van Roekel, University of Massachusetts, Amherst, Mass., as IB-41, in May, 1941, and received, after numerous bird passages, as saline suspension of tracheal mucus Mass. Lot. 220 on October 23, 1946, and having undergone eight subsequent passages both in chickens and in embryos, usual titer in eggs $10^7$, hereafter referred to as the Massachusetts type.  

* Supported in part by funds provided by Public Laws 733 (9b3) and a grant from A.D.P. Branch, A.R.S., U.S.D.A.
No. A5968, was isolated by one of us (R.E.L.) from the trachea of a two-week-old chicken from the Department of Poultry Science, University of Connecticut, kept frozen since July 17, 1951, and egg-propagated in February 1954. Every year the flock of origin was known to undergo a mild respiratory disease during the early brooding period, followed by apparently solid immunity as evidenced by significant virus neutralization (VN) titers in adults and refractivity to natural exposure, which was considered severe due to vicinity of an egg laying contest. For experimental immunization, the fifth, for VN tests the 15th, egg passage was used both with a usual titer of $10^8$, and referred to hereafter as the Connecticut type.

No. A36896, was isolated by Dr. W. R. Dunlop, University of New Hampshire, Durham, N. H., and received from him as IB DG 5, 24th lyophilized embryo passage, on February 17, 1954. For experimental immunization and VN tests, the strain had undergone three additional egg passages, with a usual titer of $10^7$.

**Antiserums**

Known susceptible chickens were inoculated intratracheally with the respective strains and maintained in isolation for three weeks, exsanguinated, and serum samples stored without preservative at $-20^\circ$C.

**Eggs**

Embryonated chicken eggs were obtained from a known IB immune flock, the only available source, and used after incubation for nine to 10 days. Previous experience in this laboratory indicated IB virus titer in eggs from a nonimmune flock was enhanced by about 1 log.

**Chickens**

For transmission studies, six to eight week-old chickens reared in isolation and known to be free from significant VN antibodies were used.

**METHODS**

**Histopathologic Methods**

Cross-cut mid portions of trachea from exsanguinated birds were fixed in Bouin’s fluid embedded in paraffin, and stained with hematoxylin-eosin.

**Virus Neutralization Test in Embryos**

Except for the egg-adapted strain (No. 66579) which had a definite mortality pattern, virus titrations were carried out by inoculating intraallantoically four eggs each with 0.2 ml of 10-fold serial dilutions of virus in Difco tryptose broth. The endpoint was determined by death or by weighing, to the nearest gram, the individual embryos on the 18th day of incubation and considering a weight of 15 grams or less as positive. The normal weight of embryos of so-called 24 ounce eggs, averaged 22 grams at 18 days.

For virus neutralization tests, 0.1 ml of double strength dilution of virus was mixed with 0.1 ml undiluted serum, inoculated immediately, and read as above.
IMMUNOLOGIC DIFFERENCES IN VIRUS STRAINS

As a rule, three virus dilutions were employed, aimed at a reduction of titer of from 10 to 1000 VN doses in a heterologous mixture, of 10,000 to 1,000,000 in a homologous mixture. In some instances the end point was not reached and the VN doses were estimated.

Virus Neutralization Test in Chickens

Virus-serum mixtures, prepared as for embryos, were inoculated intratracheally into pairs of susceptible chickens, and tracheas obtained for histologic examination two days postinoculation. Clinical signs were noted whenever objectively observable.

Cross Immunization Tests

Chickens held in isolation cabinets, were inoculated intratracheally with 0.2 ml of a virus dilution containing 100 minimum embryo infective doses (MEID), re-inoculated three weeks later, and killed two days after the last inoculation for serologic and histologic examinations.

PATHOGENESIS

The morphologic changes in the tracheal mucosa brought about by uncomplicated IB, were studied by inoculating virus strains intratracheally into groups of isolated chickens, and killing a bird for histologic and serologic examination every three days for a period of 21 days. It was found that for similar virus doses, the histopathologic response was the same for the three strains, which fact obviated the necessity of separate descriptions (7).

The normal trachea (Fig. 1) is lined by a low mucosa, uniform in thickness. The recognizable layers from the inside-out consist of a ciliated low-columnar epithelium with evenly spaced, intraepithelial glands, a cellular portion of the propria about two to three cells in depth, and an outer relatively acellular fibroelastic portion. External to the latter is a loose-meshed reticular layer, carrying the major vascular system and separating the propria from the underlying perichondrium of the cartilage.

The changes in the trachea inoculated with IB, have been found divisible into three sequential phases (7) namely the acute, reparative and immune phase. Although there is some overlapping in minor features, their principal characteristics differ widely so as to suggest different diseases to the casual observer.

In the acute phase (Fig. 2) the entire mucosa is markedly thickened, of from five to 10 times its normal depth. The lumen contains some mucofibrinous exudate with scattered heterophils, the epithelium has lost its cilia, is hypertrophied and often vacuolated, and sparsely invaded by heterophils. The intraepithelial glands have practically disappeared. The epithelium is underlaid by a zone of massive edema, containing congested capillaries and slight cellular infiltrates. The cellular portion of the propria shows moderate proliferation of mononuclear cells, chiefly histiocytes. The fibrous portion is thickened by edema.

The reparative phase (Fig. 3) gives the first indication of reduction in the pathologic thickness of the mucosa. The exudate in the lumen is diminished or absent, the epithelium, although largely without cilia, has become cuboidal or low colum-
Photosmicrographs of tracheal sections from birds inoculated with infectious bronchitis virus on same day, and examined on different days postinoculation. The cartilage extends about 1/2 inch from bottom in each figure. Hematoxylin-eosin. X115. Fig. 1.—0 days, normal. Fig. 2.—3 days, acute phase. Fig. 3.—6 days, reparative phase. Fig. 4.—18 days, immune phase.
narr, and shows a tendency to form intraepithelial glands. The cellular portion of the propria presents massive proliferation of mononuclear elements which have compressed the former edematous areas, and seemingly have overflowed into the fibrous portion of the propria.

The immune phase (Fig. 4) is characterized by return to normal of a large part of the mucosa; the cilia may not be completely restored, the regenerated epithelial cells somewhat large, and the intraepithelial glands irregularly shaped and spaced. At intervals, however, one sees bulging lymphfollicle-like aggregates in the propria, covered by thin epithelium, or inconspicuous remnants of proprial infiltrates which alterations eventually disappear.

The ordinary cycle of the lesions was for the acute phase about three days, for the reparative six to nine, and the immune phase 12 to 18 days. Modification could be brought about primarily by changing the virus dosage from the standard dose of 100 MEID in either direction. Low dosages (0.1 to 1 MEID) decreased the intensity of the acute phase, and delayed its onset and also the demonstrability of significant VN antibodies. High dosages (10⁴ to 10⁶ MEID) did not intensify the alterations above those of the 10⁴ dosage, probably because the mucosal tissue had already reached maximum reactive capacity, but high dosages advanced demonstrability of VN antibodies. Under uncomplicated conditions, the tracheal mucosa exhibited a predictable pathologic response to, as well as a remarkable recuperative power from, contact with IB.

**SEROLOGIC ANALYSIS**

*Virus Neutralization in Embryos*

The results of virus neutralization tests in chicken embryos are recorded in table 1. It will be seen that anti IB type Conn. serum neutralized over 10⁴ doses of Conn. virus, but only insignificant doses of IB Mass. and N.H. virus. Anti IB Mass. type serum in turn failed to neutralize IB Conn. type, but did so for IB Mass. and N.H. virus. Anti IB N.H. serum behaved very much like anti IB Mass. serum. The egg-adapted strain for which no effective antiserum could be prepared, was not neutralized by anti IB Conn. serum, but was fully neutralized by anti Mass. and N.H. serums.

**TABLE 1**

*Virus Neutralizing Doses in Chicken Embryos Inoculated with Mixtures of Virus and Antiserum*

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>&gt;10,000</td>
<td>10</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Mass.</td>
<td>10</td>
<td>&gt;100,000</td>
<td>&gt;10,000</td>
<td>100,000</td>
</tr>
<tr>
<td>N.H.</td>
<td>10</td>
<td>10,000</td>
<td>&gt;10,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>
TABLE 2
Histopathologic Reactions in Chickens Inoculated with Mixtures of Virus and Antiserum

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Virus</th>
<th>Conn.</th>
<th>Mass.</th>
<th>N.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>(-)</td>
<td>(+)</td>
<td>(\pm)</td>
<td>(\pm)</td>
</tr>
<tr>
<td>Mass.</td>
<td>(\pm)</td>
<td>(-)</td>
<td>(+)</td>
<td>(\pm)</td>
</tr>
<tr>
<td>N.H.</td>
<td>(\pm)</td>
<td>(-)</td>
<td>(-)</td>
<td>(\pm)</td>
</tr>
</tbody>
</table>

?-mild lesions, believed to be due to secondary infection.

Virus Neutralization in Chickens

The results are recorded in table 2. When chickens inoculated with serum-virus mixtures, were subjected to histopathologic examination two days postinoculation those inoculated with homologous mixtures failed to present lesions, except in the case of the N.H. strain, where the mild lesions observed were believed to be due to accidental contamination. In the chickens inoculated with heterologous mixtures, anti IB Conn. serum failed to protect against Mass. and N.H. virus strains, whereas the latter serums furnished protection against the respective virus strains.

Cross Immunization

The cross immunization results together with terminal VN analysis, are presented in table 3. Chickens were immunized by intratracheal inoculation of 100 MEID doses of virus and challenged three weeks later in the same manner. The results were assessed histologically and serologically. Homologous challenges failed to induce lesions, and the corresponding serums contained high levels of VN antibodies against the original immunizing virus. In parallel heterologous tests, IB Conn. type virus failed to protect against challenge with IB Mass. and N.H. virus and also failed to induce significant VN antibodies against these viruses. Mirror immunizing tests with IB Mass. type and N.H. virus and challenge with IB Conn. type virus confirmed these findings.

TABLE 3
Histopathologic Reactions and Virus-Neutralizing Doses of Immunized and Challenged Birds

<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Path.</td>
<td>VND</td>
<td>Path.</td>
</tr>
<tr>
<td>Conn.</td>
<td>(-)</td>
<td>100,000</td>
<td>(-)</td>
<td>100,000</td>
</tr>
<tr>
<td>Mass.</td>
<td>(\pm)</td>
<td>100</td>
<td>(-)</td>
<td>100,000</td>
</tr>
<tr>
<td>N.H.</td>
<td>(\pm)</td>
<td>10</td>
<td>(-)</td>
<td>100,000</td>
</tr>
</tbody>
</table>
DISCUSSION AND SUMMARY

Three widely used infectious bronchitis immunizing strains were subjected to antigenic analysis by three mirror tests, namely by virus neutralization in chicken embryos and in chickens, and by cross immunization followed by challenge. Takes in chickens were assessed by histopathologic examination of the trachea two days postinoculation and by serologic analysis for virus neutralizing antibodies.

The results by all three tests indicated the existence of at least two antigenic types of infectious bronchitis virus, in agreement with preliminary observations of Hofstad (6). In analogy with the terminology of vesicular stomatitis virus it is proposed to name strains similar to the authors' record No. A5968 infectious bronchitis virus Connecticut type, and No. 82828, Massachusetts type. A New Hampshire strain and a completely egg-adapted strain were antigenically related to the later type.

Histopathologic examination of trachea proved superior to clinical examination as a means of assessing takes. The advantages are objective observation, permanent record, availability of results within two days postinoculation, and need for few birds to be held in isolation. The method is applicable to immunity tests for infectious bronchitis on field specimens.

Both antigenic types of infectious bronchitis virus produced similar histopathologic lesions in the trachea. The pathogenesis showed a definite cycle from the acute to the reparative to the immune phase back to normal in about 21 days. In applying histopathologic methods to the diagnosis of avian respiratory diseases, the pathogenetic spectrum of the respective lesions must be taken into account.

Although the practical implications of antigenic differences in infectious bronchitis virus are not known so far, the demonstrated plurality of infectious bronchitis virus deserves consideration in the preparation and evaluation of vaccines.

REFERENCES

The chairman of this committee wishes to express appreciation to other members of the committee who willingly contributed sections of this report.

In reviewing the research work in the field of poultry diseases that has been done during the past year, one is aware that progress is being made, however slowly, in the knowledge and control of transmissible diseases of poultry. New problems, such as synovitis and ornithosis, increased in importance while others, such as hemorrhagic syndrome of chickens, decreased in incidence during the past year. In general, however, the major problems, such as leukemia, respiratory diseases, Salmonellosis, and others that have confronted us in the past are still the major problems today.

**RESPIRATORY DISEASES**

*Newcastle Disease*

Of interest during the year was the finding by the Texas workers of a highly fatal type of Newcastle disease (NCD) in the El Paso, Texas, vicinity causing a mortality of 90 to 95 percent in adult chickens. Still later Grumbles and Flowers (1) studying presumed B, NCD vaccination failures found that at least one NCD virus isolation made by Cartrite at the Gonzales Station was of a highly fatal type and that any of the B, type vaccines did not completely protect against the nervous symptoms and viremia as the mortality was approximately 20 to 30 percent of the vaccinated individuals in chickens nine weeks old. The picture was of a confusing nature in that there was a question of the efficiency of the vaccines. It was not until after considerable laboratory studies that the picture became clarified. Difficulties of this nature occurred in the Gonzales and Waco areas. The United States Department of Agriculture, Agricultural Research Service has surveyed both the Waco and Gonzales areas and there have been no recurrences of the highly fatal NCD on the same farms following depopulation of all stock and re-stocking. In one of the outbreaks the origin of the infection would seem to have been from chicks introduced from another state, which, if verified, would indicate that such vaccination difficulties are not confined to Texas. For further information on NCD, the 1956 Report of the American Veterinary Medical Association Committee on Poultry Diseases is suggested.

**INFECTIOUS BRONCHITIS**

The recent work of Hofstad (2) and Raggi (3) comparing virulent and attenuated infectious bronchitis viruses in the immunization of chickens indicates that the virulent forms of virus result in the most satisfactory immunization of flocks.
Recent attempts by Davis (4) in Texas to propagate infectious bronchitis virus in tissue culture have failed when chick embryo, muscle, heart, and chorio-allantoic membrane tissues are used. Under the same conditions Newcastle disease virus grew readily. Infectious bronchitis virus also failed to grow in HeLa cell tissue cultures under the same conditions in which Newcastle disease virus grew readily.

Vaccination of chickens against infectious bronchitis by exposure to the virus has come into widespread use in the United States in broiler as well as laying flock replacements. In general, the immunity against infectious bronchitis is satisfactory as a result of such vaccination, although some reports indicate some vaccines have poorer immunizing ability than others. All too often infectious bronchitis is blamed as the respiratory infection seen following both Newcastle disease and infectious bronchitis vaccination. The respiratory form of NCD is not uncommon following vaccination because none of our present day vaccines affords protection against respiratory involvement of the field strains of virus. These mistakes in diagnosis can only be shown through isolation of the causative virus during a respiratory disease outbreak.

**Chronic Respiratory Disease**

(CRD) continues to be a major problem for the poultry industry. Additional work is needed in elucidating the etiological factors of the CRD complex. A pleuropneumonia-like organism (PPLO) apparently plays a prominent role in the cause of the disease particularly in its chronicity (Fahey and Crawley (5). Frequently *E. coli* is found as a complicating factor in CRD (Gross) (6). The role of the so-called CRD virus isolated by Fahey and Crawley has not been definitely established. Fahey and Crawley (5) have found the CRD virus alone to cause a CRD condition in only four instances and in these cases it was of five to seven weeks duration without the usual residual symptoms. Strout et al. (7) have likewise isolated the so-called CRD virus from acute outbreaks of respiratory disease where PPLO were not involved and in which the disease lasted for only 10 days. This might indicate that the CRD virus acts similar to Newcastle disease virus and infectious bronchitis virus in activating and intensifying a latent, non-clinical PPLO infection.

The nomenclature of CRD remains unsettled. Fahey and Crawley (5) have suggested pleuropneumonia coryza for the clinical condition caused by PPLO and usually confined to the upper respiratory passages. They suggest the term CRD for the syndrome characterized by persistent signs of coughing, sneezing, rales and coryza. Edward and Freundt (8) have suggested the term *Mycoplasma gallinarum* for the PPLO of avian origin. This nomenclature will possibly influence the terminology of the disease and will render the previously suggested name of *Astercoccosis* (9) for this unsuitable.

Efforts to control the disease have been directed toward elimination of the PPLO carrier by employing the agglutination and hemagglutination-inhibition tests (Crawley and Fahey (10); Domermuth and Johnson (11); Adler et al. (12); Strout et al. (7). Some progress has been made in obtaining clean flocks indicating some degree of satisfaction with the serological tests. A pleuropneumonia-like organism isolated from pigeons (13) did not appear related to the chicken and turkey.
strains of PPLO. Gianforte et al. (14) found no serological differences in seven PPLO isolates studied. Growth of the PPLO in artificial media has been studied by various investigators (15, 16) in attempting to find a more suitable medium for isolation and antigen production. Progress has been made in this area but there is need for additional work.

Treatment of CRD with antibiotics is still being evaluated. Crawley and Fahey (10) found chlorotetracycline or oxytetracycline at 200 grams per ton level for seven to 10 weeks without effect on the course of the disease. It resulted in retardation or suppression of the HI antibody, and PPLO could still be isolated after treatment. Yamamoto and Adler (17) found differences to antibiotic sensitivity among PPLO of avian origin. In vitro studies indicated tetracycline and oxytetracycline as most active. By the in ovo method erythromycin and viridogrisein were most active.

**Infectious Sinusitis of Turkeys**

This continues to be a major problem for the turkey grower. Much of the work that has been done on Chronic Respiratory Disease is applicable to infectious sinusitis of turkeys since PPLO is believed to be the primary etiological agent. Some progress is being made in the control of this disease by using breeder flocks which are free of sinusitis. Serological tests for PPLO infection have not been carried out extensively, but should aid in the control of this disease in the future. Studies (18) of high level antibiotics against artificially induced aerosacitis in turkeys found that two tetracycline antibiotics, panmycin and terramycin, in a concentration of one part antibiotic to 630 parts of mash (4.2 percent) was highly effective in preventing artificially induced air sac infection with PPLO.

**Ornithosis**

Outbreaks of ornithosis in turkeys have been recognized in Texas since 1948 when a number of human cases traceable to processing turkeys were recognized by the Texas Public Health Department.

Recognition and isolation of the virus from turkeys first occurred in 1952, following human cases discovered in the same plant workers as that affected in 1948. In 1953 no cases traceable to turkey ornithosis were recognized; this was followed by an extensive outbreak in poultry processing workers in 1954. Only one human case was recognized in 1955 and some 30 cases and one fatality occurred in September, 1956. This peculiar epidemiology of ornithosis is very little understood at this time, although efforts to find the reservoirs in nature are being extensively studied at the Texas station.

Three turkey flocks affected with ornithosis have been recognized in September to November in Texas with one flock accounting for the 30 human cases. Since 1954 ornithosis in turkeys involving a highly pathogenic type of virus has been reported from Oregon and New Jersey. Other virus isolations have been made in California and Minnesota with the pathogenicity being of a low type.

Studies completed at the Texas Experiment Station have shown that virus of the virulent type is not transmitted through the egg to the offspring; some 6,000 turkey eggs have been assayed in such studies.
The prophylactic use of chlortetracycline at the rate of 100 grams per ton or more prevents death in highly susceptible pouls. At the rate of 400 grams per ton or more virus could not be isolated from the inoculated pouls.

In therapy studies involving both young and adult turkeys after infection was established showed that following two weeks of treatment 100, 200, 400 and 800 grams per ton of tetracycline that virus could be recovered from some individuals, but after three weeks could be recovered from pouls at the 800 grams per ton level but was not recovered from any of the adult turkeys including the surviving adult controls, thus therapy plus time is essential in the ability of adult birds to overcome the virus. Page (19) in California was unable to recover virus 42 days following infection of adult birds. It would appear that latency of the highly pathogenic ornithosis viruses is not of the significance as that reported from psittacine birds.

The indirect complement fixation serological test of turkey scra is of a confusing nature. Surveys made in Texas would indicate a high rate of infection depending on whether one regards low titers of significance and much less if only higher titers were considered. Since no virus was isolated from turkeys in 1955, it is difficult to evaluate these studies.

The pathological lesions, symptoms and mortality were studied in pouls from one week through twenty-four weeks of age. In general, the disease symptoms, lesions and mortality remains approximately 20 to 25 percent. The lesions most prominent were fibrinous pericarditis, fibrinous peritonitis, enlarged livers with a tendency for biliary stasis and covered with a fibrinous exudate, fibrinous and caseous involvements of the air sacs. Over 600 turkeys have been studied for the gross pathology of ornithosis. Fixed tissues are being studied to observe the microscopic pathology.

In studies to determine reservoirs of virus in wild birds and rodents over 900 specimens have been screened for the virulent form of ornithosis virus with negative results.

**Laryngotracheitis**

From available information this disease has decreased in most poultry producing areas due to proper management and vaccination. The disease is of rare occurrence in the Southwestern States. In the relatively free areas, there is need for continued education to prevent the introduction of vaccines which establish foci of infection. In some areas of the United States continued vaccination is necessary to avoid the losses from this disease.

Cover (20) has reported recently an outbreak of laryngotracheitis in broilers in which the mortality was negligible. Isolation of the virus and subsequent inoculation of chickens also failed to cause mortality. This is remindful of the subacute outbreaks which have occurred in Australia in recent years (21).

**Infectious Coryza**

Most sections of the country are apparently free of *Hemophilus gallinarum* infection. California however still considers this disease a problem in some flocks, and has been diagnosing the disease by cultural methods. In areas where it is difficult
to break contact between old, chronic "carriers" and new replacement stock, the disease will be difficult to eradicate.

**Fowl Cholera**

The acute septicemic form of fowl cholera is seldom encountered in the Southwest and Northeastern United States, and it is doubtful if it is a disease of widespread importance in most of the country. Reports of occurrence of the acute form of the disease from the Northwestern and North Central States reminds those concerned with diagnostic work that the disease is not to be ignored. The respiratory form of the disease is becoming less and less important, particularly in commercial poultry production, except perhaps in large caged layer operations in which the practice of assembling replacement pullets from various farms had come into use.

Of recent interest in the diagnosis of chronic fowl cholera is the report (22) of an atypical pasteurella organism isolated from chickens suffering from chronic fowl cholera. Yaw et al. (23) have studied the virulence factor of Pasteurella multocida for chickens, and have found that it is not related to the presence of a capsule, but apparently to some other cellular component.

**SALMONELLOSIS**

**Pullorum Disease**

Marked progress in eliminating pullorum disease from turkey and chicken breeding flocks through testing under voluntary programs of the National Poultry and Turkey Improvement Plans has been accomplished. The results of the program are indicated in Table I (26).

At the National Plans Conference in June, 1956, at Colorado Springs, Colorado certain phases of the pullorum control program were revised: 1) Participating hatcheries and dealers shall be designated a "National Plan Hatchery" and "National Plan Dealer"; 2) Beginning July 1, 1957 the only official antigen for the rapid whole blood plate test shall be of the polyvalent type; 3) Reactors must be submitted for bacteriological examination within 10 days from date of reading the test; 4) If other members of the Salmonella group or paracolons are isolated the Official State Agency may disqualify the flock for participation or require such ac-

<table>
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<th>Year</th>
<th>Chickens Tested Number</th>
<th>Chickens Tested Number</th>
<th>Turkeys Tested Number</th>
<th>Reactors</th>
<th>Percent</th>
<th>Reactors</th>
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<td>3,310,470</td>
<td>1,014</td>
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</table>

* For the year ending June 30 (26)
tion as is deemed necessary with respect to the infection; 5) A proposed regulation requiring banding and identification of all chickens under the NPIP caused considerable discussion but failed approval by close vote.

The committee report of 1955 (US LSA) recommended that flocks under the NPIP be properly identified (25). In some states it appeared from the discussion at plans conference that no identification was used to identify United States Pul lorum-Typhoid passed or clean flocks. The success of any disease control program is the proper identification of the flocks under the program.

It has been estimated that 50 percent of the hatcheries and 65 percent of hatching egg capacity in the United States are participating in the National Poultry Improvement Plan (26). It is hoped that all states will expand their efforts in the eradication of pullorum disease but this cannot be accomplished until 100 percent participation of the industry is obtained. There is an increased interest in the part of some states to require that all hatching eggs, chicks and poults produced in the state and likewise imported into the state be under supervision and have the classification of United States Pullorum-Typhoid Clean.

Fowl Typhoid

In some areas fowl typhoid continues to be a problem but in many areas fowl typhoid is rarely encountered. Because of the hazard of this disease to hatchery supply flocks control officials should adopt effective procedures in eliminating the disease from known infected flocks.

Paratyphoid

In some areas paratyphoid infections have been reported as serious problems in hatchery supply flocks. Williams has outlined a suggested program for the control of paratyphoid infections of poultry (27). The committee on Salmonellosis and related enteric infections of poultry of the NPIP recommended at 1956 plans conference that if a paratyphoid detection testing program was initiated in any state that the O antigen be used as screening antigen (24). If paratyphoid infection is identified in a breeding flock by bacteriological procedures that the flock be discontinued or retests be done by using both O and H antigens at 30 days intervals until a negative serological or bacteriological test was obtained.

Furazolidone has been widely accepted as an effective drug in the treatment of Salmonella infections in chicken and turkey flocks. Reports have indicated the drug may have considerable value in the treatment of chronic carriers of fowl typhoid and pullorum disease (28, 29, 30, 31). The Plans Committee recommended that breeding flocks under the NPIP program should not receive furazolidone or any other anti-salmonella drug for 60 days prior to the pullorum testing program because of the hazard of suppression of the development of agglutinins by carriers. A concentration of 0.04 percent of furazolidone in the feed for 10 days or longer is necessary to achieve this effect (24).

PARASITIC DISEASES

Coccidiosis. Cuckler and Malanga (32) studied 40 strains of allegedly resistant coccidia and revealed 43 percent to be sensitive to one or more drugs studied, 43
percent were resistant to nitrophenide, 45 percent to sulfaquinoxaline and 57 percent to nitrofurazone. Some strains were resistant to all of these antioococidial drugs, while others were resistant to two of the compounds. In some strains specific resistance was observed but cross-resistance was observed more frequently than specific resistance. Available evidence does not indicate that resistance in coccidia is at present an important practical problem. Experimentally induced resistance was demonstrated for one strain of *Eimeria acervulina* and two strains of *Eimeria tenella* after exposure to sub-optimal dosage of sulfaquinoxaline. No evidence was obtained to show that one strain of cecal coccidia exposed to sub-optimal dosage of nitrophenide, nitrofurazone or nicarbazine became less sensitive to these antioococidial agents. Nicarbazine effectively inhibited all strains of coccidia whether sensitive or resistant to other agents.

Cuckler and Malanga (33) reported nicarbazine, an equimolar complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine, to be effective in preventing coccidiosis and at the same time to permit the development of a high degree of immunity. Data was presented to show the effect of nicarbazine on development stages of *E. tenella* and *E. necatrix*. Merozoites of second generation schizonts are suppressed by the drug.

Natt and Herrick (34) studied the daily changes in body weight, percent corpuscular volume of blood, plasma volume and total blood volume of chickens infected with cecal coccidiosis. There was an appreciable decrease in body weight, percent corpuscular volume, and total blood volume. There was no change in plasma volume during the hemorrhagic phase of the disease. The decrease in blood volume resulted primarily in loss in corpuscular volume.

Levine and Herrick (35) showed that *E. tenella* reduced the ability of chickens to do muscular work. The effect was more pronounced in SCWL than in BPR chickens.

Schildt and Herrick (36) reported that *E. tenella* disturbed the motility of the digestive tract.

Edgar (37) stated that during the past three and one half years vaccine against coccidiosis has been used to immunize more than 50 million chickens throughout the United States. Vaccine is produced by the Poultry Department of the Alabama Experiment Station and released to pharmaceutical firms through the Auburn Research Foundation.

**Other Parasitic Diseases**

Vianello and Vicenzoni (38) reported piperazine citrate administered to fowls in doses of 300–400 mg. per kilogram of body weight causes complete disappearance of both mature and immature *Ascaridia*. Therapeutic doses of the drug were tolerated well.

Bradley (39) reported a considerable degree of anthelmintic value from the use of piperazine citrate in chickens. There was no noticeable alteration of feed and water consumption. Two field infected flocks were studied. One contained 15,600 eight-week-old chickens and was treated for 60 hours with 8,000 mg. of drug per gallon of drinking water. Medicated birds when slaughtered for market were found to have an average of 0.24 immature *Ascaridia galli* worms while nonmedicated
controls had an average of 6.82 mature and 2.42 immature worms of the same species. In a second flock given a smaller does (6,000 mg. per gallon of drinking water) for 24 hours at six weeks of age, medicated birds carried an average of 0.85 immature and 0.14 mature *A. galli* worms when slaughtered for market. Nonmedicated controls carried an average of 2.53 mature and 2.44 immature worms of the same species.

Nemeseri and Hodasy (40) reported that benzene may be used with good results in the treatment of ascaridiosis of fowls. The drug is not toxic in therapeutic doses. The dose of benzene for hens is 2 ml. per kilogram of body weight. It may be administered with a rubber tube attached to a syringe. Treatment may be repeated in one to two weeks.

Horton-Smith (41) reported that Furazolidone at a concentration of 0.01 to 0.02 percent w/w in the food almost wholly prevented histomoniasis induced by feeding *Heterakis* ova to turkeys when continuous medication is commenced before ova are administered. The action of Furazolidone on histomonads is inhibitory, directly and indirectly, but the protozoa proceed to multiply if medication is withdrawn or used in a very low concentration in the food. Owing to testicular changes observed in treated fowls, medication of breeding flocks is contra-indicated.

Edgar (42) reported that Di-N-Butyl Tin Dilaurate is effective in removing *Raillietina cesticillus* from chickens. The compound was effective in eliminating three other common species (*Choanotaenia infundibulum*, *Davainea proglottina* and *Hymenolopis carioca*) from laboratory infected chickens when administered in feed at 500 mg. per kilogram of feed for two to six days or by capsule at 75 to 125 mg. per bird. The compound was highly effective in removal of six common species (aforementioned plus *R. tetragona* and *Amoebotaenia sphenoides*) from field-infected hens when administered in feed at that rate of 500 mg. per kilogram of feed or as a single dose of 125 mg. by capsule per bird. No ill effect was noted from the treatment.

Tugwell (43) examined 260 birds from four groups of replacement stocks on various degrees of restricted range feed. The parasites found included *H. gallinae*, *A. galli*, *H. carioca*, *R. cesticillus*, and *C. columbae*. A general tendency existed for birds on more restricted diets to carry heavier loads of helminths. An increased worm load above that expected was evident after housing.

**AVIAN LEUKOSIS COMPLEX**

*Visceral lymphomatosis*—continues to be of major economic importance to the poultry industry. Considering the importance of this disease it is difficult to understand why more support for fundamental research in this important area is not being made available.

The propagation of the virus of visceral lymphomatosis in embryonating chicken eggs by Gentry (44) and the demonstration (45) of *in vitro* and *in vivo* neutralization of the virus of visceral lymphomatosis by serum from chickens injected with virus should aid in further studies on this disease. Burmester (46) has found the virus of visceral lymphomatosis in the saliva and feces of lymphomatosis-infected chickens and apparently normal chickens. The report of Burmester et al (47) indicating the possibility of spreading visceral lymphomatosis with live virus vac-
cines prepared from eggs from hens known to be shedders of the lymphomatosis virus emphasizes the need for vaccine producers to use care in selecting flocks which supply eggs for vaccine production.

**INFECTION SYNOVITIS**

Infectious synovitis was first recognized as a separate disease entity by Olson *et al.* (48) in West Virginia and Wills (49) in Texas in 1954. The disease has now been recognized in all major broiler producing areas and has appeared less frequently in layer flocks. Considerable attention was focused on infectious synovitis as a result of condemnations and trim outs in poultry inspection plants (50). A large percent of these trim outs were due to breast blisters not related to infectious synovitis. When breast blisters occur as a result of infectious synovitis joint involvement nearly always occurs and the breast blister contains a purulent or caseous exudate. Breast blisters from noninfectious causes contain a clear to straw-colored fluid with or without small cartilaginous granules. The fluid is occasionally bloody as a result of injury.

The cause of infectious synovitis has been classed as a large particle virus based on the susceptibility of the agent to antibiotics, the ability of the agent to pass a Sietz filter, the lack of growth in cell free media (51, 52, 53), and particle size determined by electron photomicrographs (54). Other agents produce lesions that may be confused with infectious synovitis, notably *Micrococcus sp.*, *Salmonella pullorum* (55), pleuropneumonia-like organisms (56), and fowl pox virus (57). The other agents do not produce the extreme emaciation, dehydration, splenomegaly and enlarged kidneys so frequently seen in birds with infectious synovitis (57).

Shelton *et al.* (58) compared the efficacy of chlortetracycline, oxytetracycline, tetracycline, furazolidone (NF-180) chloromycetin, magnamycin, and furadantin (NF-153) and classed the efficacy of these antibiotics in the order named.

Munro *et al.* (59) compared intermittent chlortetracycline and NF-180 and streptomycin injections. Chlortetracycline was effective only during periods of administration of the drug, NF-180 was not effective. Streptomycin injections were 84 percent effective in preventing the development of lesions when given at time of inoculation. Streptomycin injections four days after foot pad inoculation with infectious synovitis and repeated every four days was not effective in controlling the disease. Olson *et al.* (56) selected 10 cultures that were isolated from the synovial fluid of chickens. Seven of the cultures were infectious synovitis, one was a pleuropneumonia-like organism and two were unidentified viral agents other than infectious synovitis. Aureomycin 100 and 200 grams per ton was effective in controlling the disease caused by the seven strains of infectious synovitis. NF-180 200 grams per ton was fed to birds inoculated with two of seven strains and was not effective. Aureomycin was partially effective against the PPLO synovitis whereas NF-180 was not. Neither aureomycin nor NF-180 was effective in controlling the disease in birds inoculated with the two agents that were not infectious synovitis. It should be pointed out that the disease produced by the latter agents was mild and probably would not be much of a problem in broiler flocks.

The growth of viral agents in the synovial membranes of chickens needs to be
studied further. Preliminary work by Olson (57) demonstrated the growth of the fowl pox virus in the synovial membranes but not Newcastle disease, infectious bronchitis and laryngotracheitis.

ENCEPHALOMYELITIS IN PHEASANTS

Reports of naturally occurring Eastern encephalomyelitis was reported during the summer and fall of 1956 in pheasants reared in captivity in New England. This suggests the need for further work to determine the source of these infections and the importance of the problem to public health.

HEMORRHAGIC SYNDROME

The incidence of the hemorrhagic syndrome has continued to decrease. Nelson and Morris (60) produced hemorrhages in chicks supplied sulfaquinoxaline plus 0.5 and 1 mg. menodione as menodione sodium bisulfite per 100 gm. of diet. These workers stated that the clotting times were within the normal range and a closer correlation was observed in the symptoms of vitamin K deficiency and the field hemorrhagic syndrome than previously reported. It should be pointed out that the hemorrhagic syndrome has occurred in the field without the feeding of sulfaquinoxaline and without a deficiency of vitamin K. The cause of the hemorrhagic syndrome remains a mystery.

COMMITTEE RECOMMENDATIONS

Regulations for the control of poultry diseases.

Since the committee report of last year regarding recommendations for veterinary supervision of the Salmonella control program, a veterinarian has been added to the staff of the Animal Disease Eradication Branch, specifically assigned to the poultry disease section. This is a step in the right direction for an effective disease control program.

Also an advisory committee was appointed in the Department of Agriculture to establish better cooperation between the Poultry Improvement Plans, which are under the direction of the Animal and Poultry Husbandry Research Branch, and the Animal Disease Eradication Branch. However, the committee feels that continued effort should be made to bring the Salmonella control program under the supervision of the Animal Disease Eradication Branch.

At the present time the committee recommends that the livestock sanitary official of all states take a more active interest in the poultry disease control program in order that they will be able to handle the problem if and when the Salmonella control program does come within the jurisdiction of the Animal Disease Eradication Branch.

SALMONELLA TYPING LABORATORY

Dr. P. R. Edwards of the Communicable Disease Center in Georgia has for years made it possible for animal disease diagnostic laboratories to have their Salmonella and Paracolon cultures typed. This has been greatly appreciated by many laboratories. Due to the increased demand for his laboratory to type only
Salmonella of human origin he has made it known that he would like to be relieved of the additional load of typing Salmonella of animal and poultry origin. It is imperative that this typing service be continued for animal and poultry Salmonella to aid in an intelligent evaluation of our Salmonella problems. Therefore the committee would like to recommend that a laboratory be established in the Agricultural Research Service for the purpose of typing cultures of Salmonella and related organisms of animal and poultry origin.

Finally, the committee recommends that the present poultry meat inspection service be brought under the Meat Inspection Branch of the Agricultural Research Service.

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57. Olson, N. O.: Unpublished data.


FIFTY YEARS OF FEDERAL MEAT INSPECTION

C. D. VAN HOUWELING

Director, Livestock Regulatory Programs, Agricultural Research Service, United States Department of Agriculture

Federal meat inspection is like an insurance plan that begins paying dividends on the day it starts operating.

For 50 years the Federal stamp of approval on meat has given assurance to consumers that the meat was from a healthy animal and wholesome, and that the meat product was pure and accurately labeled.

For 50 years it has served the entire industry by building public confidence in a wholesome meat supply, thus contributing to a steady consumer demand.

For 50 years Federal meat inspection has helped farmers learn of disease within their herds, often early enough to prevent its spread. This has helped American producers attain world-wide eminence in the matter of livestock health.

For this assurance we are now paying a yearly premium of less than 10 cents a person. That's pretty reasonable insurance. For the cost of a 10-cent cigar, a soft drink, or a candy bar, we are getting a year's assurance of a wholesome meat supply.

When President Theodore Roosevelt signed the Act of 1906, he forecast that the Federal meat inspection service would cost no more than eight cents an animal. Today the cost is 15 cents—or about five and one half cents at the buying power of the 1906 dollar. It's a bargain any way you look at it.

Federal meat inspection actually began more than 50 years ago in the interest of protecting our foreign trade. When our cattle and hogs and their products were rejected in foreign ports—at great loss to our rapidly growing livestock industry and the economy of the country—we instituted inspection of animals and animal products for export. Although we felt that European rejections were in part a device to protect their own livestock trade, our inspection served to break down restrictions in some countries, and to create confidence abroad in our exports.

About this time, the public began to clamor for inspection of meat for domestic consumption. Congressional investigations and the muckraking novel, “The Jungle,” focused a spotlight on the insanitary conditions and methods of meat packing plants. Although the charges in “The Jungle” were later shown to be exaggerated, the furor had been created. The Congress responded to public demand by passage of the Meat Inspection Act of 1906. This was just 10 years after the United States Livestock Sanitary Association was founded.

Except for minor changes, that basic law has been found good and today is the same as in 1906. Only the facilities for securing a “clean and wholesome meat supply” have changed to keep up with new developments.

Meat inspection didn’t just “grewed,” like Topsy. Its development has been steady and systematic. Fifty years ago, Federal inspection was being conducted in 163 plants in 58 cities. Today, inspectors work in about 1200 plants in nearly 500 cities and towns.
A look at a map helps you visualize the extent of the growth that has taken place. Today, the plants are concentrated in areas of high livestock production and near major marketing centers. About 80 percent of all red meat marketed passes through these plants and carries the Federal stamp of approval. The rest is marketed under State or local supervision.

Last year, more than 100 million carcasses were Federally inspected. Something like 800 carcasses were inspected every minute of every working day. That is a tremendous workload, and a tremendous responsibility for the veterinarians in the Federal service.

The importance of their work is borne home to us when we consider how definitely we are a nation of meat-eaters. During this past year, it is estimated that we ate an average of 162 pounds of red meat per person. That's the equivalent of 325 home-made hamburgers, 165 pork chops, 20 veal cutlets, and one small leg of lamb.

An idea of the gain in slaughter under Federal inspection is more apparent when we consider the various animals.

During the past 50 years, the number of cattle slaughtered under Federal inspection increased more than two and one half times—from 7.6 million in 1907 to 19.6 million in 1956.

During this period, slaughter of calves under Federal inspection multiplied about four and one half times—from 1.7 million calves in 1907 to 7.6 million in 1956.

Hog slaughter during this period more than doubled. In 1907, some 31.8 million hogs were slaughtered under Federal inspection. In 1956, the number had increased to 66.7 million.

The same kind of story is true for lambs and sheep. In 1907, some 9.6 million lambs and sheep were slaughtered under Federal inspection, as compared with 14.2 million in 1956.

At the same time, the amount of meat and meat products processed and prepared under Federal supervision quadrupled. The many new food products, including frozen meat dishes for quick home preparation, have brought the processed foods prepared under Federal inspection up from five billion pounds in 1907 to about 20 billion pounds in 1956.

Let's take a look at a few specific items. The amount of cured meat, processed under Federal inspection, increased from 2.4 billion pounds in 1907 to 3.9 billion pounds in 1956. In 1956, more than five times as much sausage, and more than twice as much lard, was produced under Government supervision as in 1907.

This summarizes briefly 50 years of growth of the meat inspection service—from the number of plants and locations to the volume of business. Now, let's review what the meat inspection service does.

Our activities may be summarized like this:

(1) Supervision of the sanitation of the plants.
(2) Inspection of animals before slaughter to eliminate those unfit for food.
(3) Careful examination of the head, glands, internal organs, and carcass of each animal immediately after slaughter.
(4) Inspection of processing, such as curing, canning, freezing of meat, making sausages and similar products.
(5) Disposal of condemned material.
(6) Supervision of the composition, marking and labeling of meat and meat food products.

(7) Maintaining laboratories to obtain chemical, bacteriological, pathological, and zoological information needed by inspectors.

(8) Certification system for export meat to comply with requirements of foreign countries and consignees.

(9) Import meat controls consisting of review of inspection controls of country of origin, approved certificate arrangement and inspection at time of entry.

These activities require that meat inspectors have a highly specialized knowledge of the basic veterinary medicine, as well as related fields. They must have a thorough knowledge of anatomy, pathology, physiology, parasitology, and bacteriology. Inspectors must give these subjects—with the exception of anatomy—a specialized food control application. In addition, meat inspectors must learn all they can about the biology, chemistry, and enzymology of muscle, fat, and connective tissue as meat. They must do this to keep abreast of new methods and new products developed in the industry, and to better evaluate their effects.

As disease control officials we have always recognized and stressed the importance of meat inspection in discovering and controlling disease. The success of these disease eradication programs and the role that meat inspection has played in disease eradication is reflected in the progressively lower percentage of animals that are found unfit for food. Today, only about one-fourth of one percent of the animals examined by Federal inspectors are found unfit for food.

The ante mortem and post mortem examinations are the keystones of the program. However, the law covers considerably more than considerations of human health. The law grew out of consumer demand, and it recognizes those demands and translates them into practices.

The consumer demands normal, clean meat tissue. Normal means more than that the product will not induce sickness in man. There are many unsavory conditions affecting animals which are not transmissible to man. But consumers insist that all of these loathsome conditions be eliminated from their food supply. We cannot tolerate the inclusion of filth—even though sterilized—in our food. Therefore the consumer protection provided under the Federal Meat Inspection Law goes beyond the health issues to assure the consumer of the observance of common cleanliness and decency in the production and distribution of his meat.

The consumer is protected also against adulteration. He is protected against mislabeling. He is protected against the use of improper methods in the production of meat food products.

This year we are celebrating the 50th anniversary of the Meat Inspection Act. Over the years, protection offered by the Meat Inspection program has served to build confidence on the part of the consumer in the meat he buys. This confidence rests firmly on the integrity of the dedicated men behind the scenes who have worked tirelessly to maintain high standards of quality. The Federal stamp of approval is dependable because of the strict and practical regulations, the high caliber of the inspection staff, and the full cooperation of all concerned.
REPORT OF COMMITTEE ON PUBLIC HEALTH

Dr. W. L. Bendix, Richmond, Virginia, Chairman; Dr. R. E. Willie, Washington, D. C.; Dr. A. K. Merriman, Springfield, Illinois; Dr. Oscar Sussman, Trenton, New Jersey; Dr. F. B. Wheeler, Baton Rouge, Louisiana; Dr. J. H. Steele, Atlanta, Georgia; Dr. F. P. Wilcox, Los Angeles, California

PSITTACOSIS

Psittacosis, also known as ornithosis has been diagnosed in a wide variety of birds (including turkeys) in the United States. Virus isolations have been made from turkey flocks in Texas, New Jersey, Michigan, Minnesota, California, Oregon and Virginia. Human illness has been associated with this disease in Texas, New Jersey, Oregon and Virginia.

Effective procedures for controlling this disease have been developed. These measures were worked out by the scientists of the nation in cooperation with industry groups and all other interested segments of our country. It is believed that these measures are effective if properly executed and we, therefore, recommend to all states that they be adopted and effectively enforced where this disease appears.

RABIES

Through the cooperation of state and federal veterinary regulatory officials, state and federal veterinary public health officials and citizen groups it appears that progress is being demonstrated in the eradication of rabies. We are a long way from our goal but in so far as the dog is concerned we can report some improvement in the situation.

In the areas of the United States where rabies in wildlife is a problem the situation if anything is worsening. Reports indicate that in these sections canine rabies has been reduced, and in some sections reduced drastically. At the same time this infection in wildlife has remained constant or increased. The Committee wishes to call to the attention of this Association, and the Nation, the fact that merely transferring the reservoir of infection from one species of animal to another is neither eradication nor control. It shows a serious weakness in the allover program and must be corrected.

WHOLESOme POULTRY

This Committee called your attention last year to the need for, and the great interest in, the inspection for wholesomeness of poultry and poultry products offered as items of human food. This Association indorsed the recommendation of the Committee that the United States Public Health Service recommended ordinances be adopted at local and state levels, and that the Federal Meat Inspection Act of 1906 be further amended to include poultry and poultry products offered in interstate and foreign commerce.

The matter of poultry inspection has been given a complete hearing before the Nation. The second session of the 84th Congress was bombarded with prop-
agenda, demands and pressures from a great many sources to do something about this situation. A great many bills were introduced aimed at providing inspection for wholesomeness. The Amalgamated Meat Cutters & Butcher Workmen of America lead off with a bill to establish a new poultry inspection service in the Food & Drug Administration. The Food & Drug Administration readily admitted its support of the measure and the great need for such a service but declined to have it placed under their jurisdiction. The Food & Drug Administration recommended that it be placed in the hands of the United States Department of Agriculture. The American Veterinary Medical Association and the United States Livestock Sanitary Association joined in an attempt to secure an amendment to the Meat Inspection Act of 1906, and actively supported legislation designed to accomplish this end.

The Institute of American Poultry Industries, drew up, introduced and supported a bill that provided for some inspection but was basically a marketing bill which in your Committee's opinion was a bad bill. Your Committee bases its opinion on the fact that poultry inspection is a service for the protection of the consumer, and for those who work in the processing and packing industry. The Institute Bill, in the opinion of the Committee, was designed primarily to help the processors and packers with their marketing problems and the inspection provisions of this bill were of secondary importance. This bill was actively opposed by the United States Livestock Sanitary Association.

The poultry industry, both processors and growers, were unable to agree on what they wanted or would support. Some segments of the industry followed the Institute, some segments followed the United States Livestock Sanitary Association and the American Veterinary Medical Association. Some followed neither, and attempted to shrug off the whole idea as unnecessary and foolish.

A great mass of testimony was given by a great many organizations. The Agricultural Marketing Service Voluntary Inspection Program came in for a great deal of abuse. Overwhelmingly the groups testimony before the conference was critical of this service, and the Committee itself voiced criticism of the Department of Agriculture for failing to call attention to this need prior to this year, and for other reasons. (Senate document No. 129—84th Congress, 2nd Session.)

The Congress took no action and adjourned leaving all of the Poultry Inspection Bills to die. Something was accomplished, however. Everybody interested had a chance to be heard. Some of the testimony was restrained and factual, some of it was heated and almost hysterical, some of it was sense, and some of it was nonsense. These facts, however, emerged: The consumers of this Nation need and are entitled to inspection for wholesomeness both anti-mortem and post-mortem on the poultry and poultry products that are offered. The Congress must provide such a service on these products offered in interstate and foreign commerce. From the standpoint of logic, knowhow, experience and a long record of sound efficient operation the Meat Inspection Branch of the United States Department of Agriculture is the place to put this service. We can no longer compromise with this matter, and this Committee therefore recommends that this Association actively pursue this course in the 85th Congress.
RINGWORM

Apart from their economic and cosmetic implications, animal ringworm infections constitute a definite public health problem. Recognizing the public health importance of the cutaneous mycoses of lower animals and the many voids in our overall knowledge of these infections, the Communicable Disease Center inaugurated, in August 1953, a comprehensive animal ringworm study. An integral part of this study is a survey of domestic and wild animals for the presence of dermatophyte species. Veterinarians from every section of the country are participating in this survey by submitting for mycologic study, clinical materials from animals with cutaneous abnormalities.

During the period of September 1955 to July 1956, a total of 770 domestic animal hair specimens were submitted for mycologic examination by veterinarians from 29 states. Of the total number cultured, 225 (29.2) were found to be positive for dermatophytes. Ringworm fungi were recovered from animals from every section of the country. These data show that animal ringworm is both common and widespread in this country.

The four principal species of dermatophytes found to infect animals are: *Microsporum canis*, *M. gypseum*, *Trichophyton verrucosum*, and *T. mentagrophytes*. *M. canis* is the organism most commonly found to produce ringworm in cats, dogs and monkeys. *M. gypseum* is the only dermatophyte found to occur in nature (soil) as a saprophyte. This fungus is the second most common organism responsible for canine ringworm. Infections have also been found in cats and the horse and wild rodents. *T. verrucosum* is the principal etiologic agent of ringworm in cattle. *T. mentagrophytes* has been recovered from a long list of domestic and wild animals. This fungus has been recovered from dogs, cats, horses and guinea pigs. In these domesticated animals definite skin lesions were observed. Recoveries of *T. mentagrophytes* were made from a variety of wild rodents and from larger mammalian species. These wild animal hosts did not manifest any cutaneous abnormalities. Apparently they serve as carriers of this fungus.

During the period a total of 44 reports of ringworm outbreaks involving both animals and human contacts were received by the Communicable Disease Center. The dermatophytes involved were *M. canis* in 37 instances; *M. gypseum* in one case; *T. verrucosum* in one episode; *T. mentagrophytes* in one case; and, other dermatophytes in four instances.

STATUS OF PARASITIC DISEASES OF ANIMALS COMMUNICABLE TO MAN

*Toxoplasmosis*—Ubiquitous, true prevalence not yet known due to difficulty in diagnosis. Role of animals has not yet been definitely established.

*Trichinosis*—is most prevalent, damage, as far as morbidity and mortality are concerned, not well known due to difficulty in diagnosis of light infections. Epidemics limited to Winter and early Spring, mainly among foreign born population.

*Visceral Larva Migrans*—Limited to children in early childhood. True prevalence not yet known due to difficulty in diagnosis. Indirect evidence suggests high prevalence rate in young children, especially in South Eastern United States. Amount of damage will depend upon location of larvae in body and number of parasites.

Taenia Soleum—Rare in humans in United States.

Taenia saginata—Prevalence low, associate primarily with migration of Mexican emigrant labor into South Western United States.

Dipylidium caninum—Uncommon, has limited pathogenesis in humans.

Hymenolepis diminuta—Moderate prevalence, has limited pathogenesis, no specific area distribution.

LEPTOSPIROSIS

During the past decade much progress has been made in the development and standardization of laboratory techniques for the diagnosis of leptospirosis and classification of Leptospira. Similarly, worthy contributions have been made toward the problem of therapy and control of the disease. However, spread of the disease in domestic animals has been much more rapid than the development of diagnostic and control procedures so that a solution to these urgent problems has by no means been reached.

In considering avenues of approach to the problem it should be remembered that the best specificity concept of certain leptospiral serotypes no longer holds true. For example, L. canicola, originally associated with dogs, is known now to infect cattle, swine, other animals and humans. Recently L. pomona, the type associated with swine and cattle, has been found in striped skunks, and raccoons (McKeever et al.) and a striped field mouse (Borg-Petersen and Fennestad, JAVMA. 128: 204, 1956). L. ballum, known to infect mice and men has been found in opossums (Yager et al. Proc. Soc. Exp. Biol. Med. 84: 589, 1953). Thus it is apparent that further studies on the role of wildlife in the epizootiology of leptospirosis should be made.

For the prevention of bovine leptospirosis, several vaccines against L. pomona have been produced with encouraging results. With the knowledge that serotypes other than L. pomona cause leptospirosis in cattle the preparation of a polyvalent vaccine should be considered.

Of utmost importance in any control program is the availability of laboratory diagnostic services. Many of the veterinary diagnostic laboratories now perform serological tests with leptospiral antigens but the number is by no means adequate.

An alarming problem in the laboratory diagnosis of leptospirosis is the apparent dependence being placed upon darkfield examinations. In recent months several reports have appeared in the literature (JAVMA 128: 601, 1956; Vet. Med. 51: 385, 1956) where diagnosis in dogs and horses was based upon darkfield examination of blood and/or urine. Further reports have been published in which darkfield examination of urine was used to determine the effect of antibiotics in experimental leptospirosis. It is well known that leptospires are not always present in blood or urine in sufficient numbers to be detected in darkfield.

In an effort to eliminate publication of data which serves only to confuse and mislead the inexperienced, all articles published should be reviewed by someone highly experienced in the field.
INTRODUCTION

The history of rabies is well-documented and will not be discussed in detail. Prior to Pasteur's classic demonstration of the antigenicity of antirabic vaccine of rabbit cord origin there was no method of protecting man or beast following exposure to a rabid animal. Until 1949 Pasteur's method was followed in principle except for the substitution of brain for cord as the substrate for propagation of the virus and for modifications in methods of preparation and killing or attenuation of the virus. All antirabic vaccine of brain tissue origin employed in animal prophylaxis in this country from 1922 to 1949 contained killed virus. Killed virus vaccine produces a relatively short and uncertain immunity necessitating annual inoculation. This is a very decided handicap on a long-time basis in mass dog immunization.

THE BIOLOGY OF RABIES

Tremendous advances have been made over the past few decades in the control of many infectious diseases of man and animals. Nevertheless, no major disease has been brought under control on a voluntary fee or revenue plan whether the recipient of the fee has been a veterinarian, physician or an agency of government. In some states, inoculations to protect children are a mandatory prerequisite to entrance to school. In most states, through the activities of state and local health departments, physicians or nurses conduct both school and health department clinics where these inoculations are administered free to all regardless of ability to pay. Any who wish to go to their personal physician for these inoculations are free to do so. The result is that many infectious diseases which were commonplace a few decades ago and for which good immunizing procedures are available are rare today.

The control of bovine tuberculosis has been hailed by the medical profession as one of the greatest contributions ever made to human health, particularly that of children. This was accomplished by free compulsory state and nationwide tuberculin testing of all cattle in the United States. The present brucellosis eradication program is a re-application of the same procedure. The question thus arises, how can free inoculation of millionaire business man Joe Doak's cattle be private enterprise at its finest and free inoculation of millionaire business man Joe Doak's dogs be socialism when both are done in the public interest? Either, both are admissible procedures of democratic government or both are violations of fundamental principles.

IMMUNIZATION AGAINST RABIES

Two recent advances, living attenuated virus antirabic vaccine of chick embryo origin and hyperimmune serum, have radically changed our previous concepts of methods of prophylaxis, control and possibly eradication of rabies.
It had long been felt that a living attenuated virus antirabic vaccine which did not reproduce the disease would confer a relatively longer immunity following one injection, and would be greatly superior to the killed virus product of brain tissue origin.

The development of living attenuated virus antirabic vaccine of chick embryo origin was announced by Dr. Herald Cox of Lederle Laboratories in the fall of 1948. The Flury strain of virus employed was isolated from the brain of a child which died in Georgia. By repeated intracerebral passage in day-old chicks this strain had become adapted to an avian environment. It was then inoculated into the yolk sac of the chicken embryo where it was found to reproduce luxuriantly in all the tissues and fluids of the embryo, thus offering an ideal medium for propagation of the virus. After sufficient embryo passages to attain the degree of attenuation required for safety, the virus retained adequate antigenicity for vaccine production.

Since chick embryo vaccine has practically no brain tissue, it is free of the danger of paralytic reactions due to sensitivity to brain tissue so common in dogs and man. That it will confer satisfactory immunity for at least four years has been confirmed by mass state-county dog inoculation programs conducted in Georgia from 1952 to date. This obviates the necessity of annual inoculation of dogs, a fact of great importance since the increasing apathy or resistance on the part of Georgia
dog owners to the nuisance and expense of annual inoculation of their dogs has be-
come an increasing concern to public health authorities.

The other major advance was the development of hyperimmune serum for hu-
man prophylaxis. This product was approved for field trial by the Georgia State
Department of Public Health in 1947 on the basis that it would be used in cases
of severe face or hand bites where the offending animal had proven rabies or when
the disease could not be excluded by history. However, one cannot experiment with
human beings, therefore, antirabic vaccine was used as indicated in conjunction
with serum, which made it difficult to evaluate the product. However, it is shown in
Graphs II and III that in the years prior to the use of hyperimmune serum there
were four or five deaths annually despite treatment whereas in the years of its
use there have been no deaths in which prophylactic measures, either vaccine or
serum, were used. This would indicate definite protective value. A total of 74
people have received injections of hyperimmune serum to date.

When serum is injected intramuscularly the protective antibodies present are
carried to the tissues almost immediately. The passive immunity associated with
serum decreases quite rapidly and is negligible after 15–20 days. Vaccine requires
15–20 days for the body tissues to produce antibodies of a protective titer, thus
serum bridges the gap between the time of exposure and that time necessary for
antibody production following administration of vaccine.

Hyperimmune serum is prepared from horse serum. If the proposed recipient
should be sensitive to horse serum as the result of previous injections of tetanus or

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**Graph III**

**Distribution of Antirabic Treatments**

- Human Deaths
- No Antirabic Treatment
- During or After Treatment

State Board of Health Laboratory

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diphtheria antitoxin, more or less severe anaphylactic reactions may be expected following injection of hyperimmune serum. An accurate history with respect to previous exposure to horse serum is important.

Rabies control and eventual eradication is going to be difficult but is not insurmountable. Fortunately the disease is spread in nature only through contamination of a fresh wound with virus bearing saliva from a rabid animal and not by contaminated food, water, inert material or casual contact. The problem is still extremely complex due to the fact that all warm-blooded animals are susceptible, particularly dogs and wildlife. In Georgia, and this is true of much of the southeast where there is a large dog and wildlife population, there are numerous varying sized tracts of forested or partially forested lands grown up with underbrush, with sawdust piles, swamps and streams which provide ideal food and cover conditions for wildlife. Much of this country is heavily populated with fox (grey and red), raccoons, bobcats, skunks, opossum and others.

Diseases and food supply, not human activities, appear to be the limiting factors on population. When any species becomes numerous enough to provide contact and intermingling, infectious and parasitic diseases reduce the population. According to reports the extensive rabies epidemics in wildlife in 1944, 1945 and 1946 reduced the fox population almost to extinction in much of south Georgia.

If left alone nature automatically maintains an equilibrium. This, plus a fairly good dog immunization program, is probably responsible for an abrupt decrease of rabies in the southeast section of the state noted by 1950-1951. A good wildlife control program concentrated in an infected area assists and hastens nature toward attaining a minimum population level at which an infectious disease such as rabies cannot maintain itself.

**Rabies in Dogs**

We estimate roughly that there are about 600,000 dogs in Georgia. For practical field control of rabies in dogs these animals may be divided into four classifications: household pets; sporting or working dogs; owned but free roaming dogs; and wild dogs.

Household pets have considerable sentimental and actual value, are usually confined to the house or owner's premises and are taken to a veterinarian regularly for the customary inoculations including rabies. The possibility of contact with roaming stray dogs is at a minimum but if contact with a rabid animal should occur, the majority already have the protection that antirabic vaccine confers and clinical rabies is relatively rare in this group. Most of these dogs are inoculated at a veterinary hospital and only a very small minority are presented at organized clinics, free or otherwise.

Many owners maintain packs of dogs which are used for hunting foxes, birds, rabbits and raccoons. These animals are usually confined on the owner's premises except while hunting when they roam the fields, swamps and woodlots in search of game. Fox hounds are customarily turned loose in packs at night and range over a wide area for hours or even a day or two entirely free of control by the owner. In a rabies area contact with a rabid dog, fox or other animal can be expected, and because of their value and the fact that the owner is usually rabies conscious,
a majority of these animals have been immunized against rabies. However, it is not unusual for large packs of hunting dogs which have never or rarely had protective inoculations to be presented at clinics.

Many farmers maintain one or two good dogs for policing the premises and working with livestock. These dogs are free to roam although they usually stay close to or on the owner's premises. They will fight off any foreign animal and are therefore frequently subject to exposure. Probably not over 50 percent of this group is under veterinary supervision and receive protective inoculations regularly.

Both the sporting and working groups are at a considerable hazard and in the past rabies was not unusual among them.

Roaming or stray dogs are our greatest menace. Many have no owner and roam at large securing food and shelter where they can. Even if someone claims ownership the dogs receive limited or no attention and are rarely if ever taken to a veterinarian. Their continued existence is based on survival of the fittest. The roaming stray dog is a real problem to dog wardens and rabies control officials. They upset garbage cans, damage flower beds, engage in fights, and if a female is in season, range over a wide area in packs in pursuit of amorous experiences.

This least immunized class of dogs is most likely to be exposed to a rabid animal, and if the disease develops is free to roam, biting other dogs and people until destroyed or until death intervenes. These dogs are smart—they learn to recognize wardens and dog trucks and are difficult to catch. Dog wardens are not popular and residents frequently get the dogs off the street when the dog truck appears, then turn them loose to roam again. Probably 75 percent of our rabid dogs fall in this group.

Rigid enforcement of dog licensing with police action to force rabies inoculation, as a prerequisite to securing a license, can do much to correct this situation. Districts operating without the assistance of license regulation and with only fair or no warden activity, find that free inoculation clinics are the best alternative in infected areas. For example, local veterinarians usually hold clinics throughout a county in the spring or following reports of rabid animals and this was done in Clayton County, Georgia this year when rabies began to occur in dogs, with about 800 dogs brought in for inoculation. However, control of the outbreak was not achieved and a free state-county inoculation program was conducted with 2,263 dogs and 127 cats presented for inoculation or almost three times the number at the previous private clinics. Essentially similar situations have been encountered many times during the course of the rabies control program.

There are many packs of wild dogs numbering 30-40 to a pack in Georgia. They feed at garbage dumps outside cities and towns and destroy wild game, farm poultry, even calves or heifers and young pigs. They are under no control whatever and are never inoculated against disease. If they become too much of a nuisance county authorities are inspired to trap or poison the pack.

RABIES IN OTHER ANIMALS

A much more subtle problem is created by the recently demonstrated presence of rabies in native insectivorous bats. Positive bats have been reported in 13 states
and it is evident that the disease is widely distributed throughout the United States in these animals. To date we have had four rabid bats in Georgia, one of which attacked a child in daylight. Two were found in Atlanta, an area which has been free of dog rabies for over a year.

On the basis of present knowledge, one cannot evaluate the significance of rabies in these mammals. It seems probable that migratory insectivorous bats originally acquired the infection from vampire bats in Mexico or South America and carried it back to their summer home to infect other bats thus spreading the infection from colony to colony and region to region. It is possible, indeed probable, the bat virus may have become partially attenuated by repeated passage through these animals. A woman in Texas was reported to have died from rabies following an attack by a bat and more recently an entomologist working with bats appears to have contracted rabies from abrasions received while handling them. These are the only reports of transmission of rabies to man or other animals by insectivorous bats. Burns and associates (1) have conducted a very thorough study of rabies in insectivorous bats, and F. Camargo N. (2) has described the disease in vampire bats. For the most recent and comprehensive information reference should be made to these reports.

RURAL AND URBAN DIFFERENCES IN RABIES CONTROL

Tierkel (3) has reported the excellent rabies control programs which have been conducted in ten of our major cities. In Georgia conditions with respect to dogs and rabies control in the counties containing the principal cities such as Atlanta, Macon, Columbus, Albany, Augusta, Rome and Savannah are quite similar to those reported by Tierkel but are vastly different from those prevailing in most of the remaining 152 counties.

In all our major cities in Georgia there is a combined county-city health department. These departments are adequately financed and all have rabies control staffs with a dog pound, a dog warden with one or more dog trucks and all operate under dog ordinances which are in effect laws. If funds are needed over the ordinary operating budget to use in an emergency these must be voted by the Board of Commissioners of Roads and Revenue. In a county with a major city, administrative officials are usually not subject to small pressure groups. Public opinion is guided by daily papers, radio and television stations. Officials in populous counties can to a large extent ignore resistance by groups whose appeal is not general.

Rural counties usually have one weekly newspaper, and the sheriff is the law enforcement officer. Many have no local radio or television facilities. In rural counties administrative officials are close to the people and are responsive even to small pressure groups, particularly if the group is organized and speaks and votes as a unit. Rural counties frequently have very limited operating funds and officials must be sensitive to adverse criticism for increasing a departmental budget even in an emergency. In a close election a family group or a quasi-political association can swing an election. Let us not be critical; elective officials face the same problem everywhere, even you and I.
RABIES LEGISLATION

It is clear that rabies control to be equally effective in rural and urban areas must be beyond the financial control of local bodies. This brings up serious questions. Should we camouflage rabies control as a revenue producing measure in the form of a dog tax? Could the county or state use dog tax money to support rabies control in wildlife? Would it not be better to enact legislation giving a state agency authority to undertake such measures as are necessary to eradicate the disease including declaration and enforcement of local quarantine, elimination of stray dogs and compulsory inoculation of all dogs, with or without fees, in the quarantine area? Authority to reduce the wildlife population as rapidly as possible in the infected area would be needed. Such legislation should provide for a budget to enable the control officer or agency to take appropriate control measures immediately upon the report of a case of rabies in a given area. This would obviate the necessity of spending weeks or even months trying to convince the local authorities that they should do something as is now the case. Legislation incorporating the measures outlined above was submitted to the Georgia legislature in January, 1954. It passed the House with four dissenting votes but was defeated in the Senate by a small outside pressure group.

PRESENT PROGRAM

The Georgia Department of Public Health and the United States Fish and Wildlife Service have attempted to control rabies by encouraging or assisting in the immunization of dogs throughout the state with major emphasis on known infected areas and reduction of wildlife, particularly foxes, in areas where indicated. Quarantine has been employed occasionally by local authorities. Control and destruction of stray dogs by erection of dog pounds and the employment of dog wardens have been encouraged on the local level. Of the 159 counties in the State only twelve have efficient dog pounds. Except in state-county assistance programs, veterinarians use the vaccine of their choice.

An educational program by use of bulletins, educational pamphlets, articles in the press, radio and television has been pursued continuously. The personnel of the regional health offices, the local county and city health offices, local veterinarians and elective county personnel have carried the bulk of the work load. The central office has very little actual authority but acts in an advisory and organization capacity with, beginning in 1952, financial assistance to the counties to encourage control activities. Financial assistance is limited to furnishing chick embryo vaccine and compensation to veterinarians for administering the vaccine and matching funds and traps in wildlife control programs. Financial assistance is offered only in known infected counties.

The State Rabies Act requires among other things the annual inoculation of all dogs in the state with a maximum fee of $1.00 per dog; provides that the county boards of health have full and sole responsibility for enforcement of the act and to appoint rabies inspectors and erect dog pounds. It was one of the first specific rabies control laws, a pioneer, and in theory a good law. It is, however, inflexible
and has no provisions for adoption of new methods and procedures indicated by experience in the field. Some sections are impracticable and therefore unenforceable.

In spite of the "compulsory" features of the act, compliance by dog owners and county boards of health has been with very few exceptions entirely voluntary. A few municipalities and counties have administered the act very effectively by enforcing dog licensing through local police action. In most of these areas, a determined individual, be he the local veterinarian, county health officer or sanitarian, has supplied the initiative and drive to motivate and maintain the objective.

Wildlife is under the control of the State Game and Fish Commission. Rabies control in these animals has been the function of the Federal Fish and Wildlife Service, United States Department of the Interior, with the sanction of the State Game and Fish Commission. Participation by the counties is voluntary.

In practice, over the major portion of the state, dog owners may comply with the annual inoculation provision of the act or ignore it with impunity. Legal action is rarely attempted. Enforcement of the act is vested in the county boards of health, which consists by law of the chairman of the county commissioner of roads and revenue, the county superintendent of schools and a local practicing physician.

Financial assistance to the counties has been made possible by liberal interpretation of the context and evident intent of the law by the State Attorney General's office. However, we have accomplished most through education and persuasion with the assistance of many people who have the desire and faith that rabies can be eradicated and by county-wide free dog inoculation programs with chick embryo vaccine.

As you may note in Map No. I, the entire state could be considered to be infected in 1946. Rabies occurred predominantly in dogs in the rolling or mountainous northern part of the state and in foxes and domestic animals in the south parts. It occurred in both foxes and dogs in the central area.

Practicing veterinarians, or in some counties, laymen were authorized to inoculate dogs and customarily held clinics at stores, county schools and churches throughout the state. Compliance as a whole was good and about 250,000 dogs were inoculated annually in 1946, 1947 and 1948. Although morbidity and mortality report cards were sent out to all veterinarians and other participants only 25 to 30 percent responded, therefore accurate figures cannot be given.

The result, however, was quite satisfactory in the south central part of the state, due we believe to two factors, enough dogs were immunized to present a fairly satisfactory immune barrier to an epidemic in dogs, and rabies killed such a mass of the wildlife population that the disease literally died out from lack of sufficient susceptible animals to maintain an epidemic. During this time one of us (Canup) was very active in assisting infected counties with wildlife control programs toward reducing the infection in these animals to a reasonably controlled status. The disease has not been completely eradicated as evidenced by more or less isolated cases in dogs, foxes and cattle and an occasional local epidemic in foxes.

During the period 1944–1948 losses of domestic animals, particularly cattle, horses and mules were severe.

It was and is a practical impossibility to determine the actual number of rabies
cases in any species. Dependence can only be placed upon laboratory examination of brain material plus information gained in the field. It is expensive and inconvenient to ship or personally transport animal heads to one of our laboratories, at Atlanta, Macon, Albany, Waycross, Columbus, Savannah and Augusta. In practice the bulk of the heads submitted for laboratory examination are the result of hu-
man exposure to a clinically or suspicious rabid animal, where some animal was destroyed after being observed among domestic animals or for a confirmatory diagnosis. Unless there is some special need or interest heads are rarely submitted. During an epidemic the number of laboratory confirmed positive cases represent only a small percent of the actual number of cases. In non-epidemic periods much information is necessarily derived from the experience of local health personnel and practicing veterinarians.

By 1949 and 1950 a decided decrease in the number of dogs immunized annually was noted. Several factors apparently were involved. There was general apathy and indifference. Many persons obviously felt that it wasn't worth the time and expense involved to stop work and take his dog or dogs to a clinic or veterinarian's office. Frequently officials made threats of punishment for non-compliance with the law, then did nothing thus creating contempt of the law.

**EXPERIMENTAL STUDY OF CHICK EMBRYO VACCINE**

After a thorough study of the laboratory protocols pertaining to the safety and antigenicity of chick embryo vaccine, an experimental study was approved by the Georgia Department of Public Health in January, 1949 using LEP (approximately 70 chick embryo passage) vaccine. The first dogs were inoculated May 11. In October, free mass inoculation of dogs in Jasper County was conducted with over 1700 dogs presented. In 1950 chick embryo vaccine was furnished to the local veterinarian in adjacent Morgan County for use on a fee basis. These two counties were in an area of endemic dog and fox rabies. A few other veterinarians used a limited amount of the vaccine in their private practice.

All inoculated dogs were tattooed in the ear for identification and all dog owners were requested not to have their dogs re-inoculated until advised. Approximately 10,000 dogs were inoculated in endemic areas in 1949 and 1950. All reports of suspicious nature among the inoculated animals were investigated. Seven inoculated dogs developed furious rabies, were Negri positive and virus was isolated from the salivary glands of four, indicating that they were infected with street virus and that the vaccine did not offer satisfactory protection. Coincident with the occurrence of these breaks potency tests were conducted and the virus in some lots of vaccine was found to be either dead or low in potency. All such lots were destroyed. Very little was done with chick embryo vaccine in 1951 pending further observation of inoculated dogs and satisfactory stabilization of the virus.

Early in 1949 four herds of dairy cattle were inoculated experimentally with LEP chick embryo vaccine. In one herd 15 cattle, adults and calves, died with atypical rabies. Whether rabies was due to street virus which was partially blocked or interfered with by the vaccine or the virus was too virulent for use in cattle has never been determined satisfactorily (4). At any rate further work with cattle immunization using LEP vaccine was discontinued pending further study of the vaccine. A challenge experiment to determine the safety and antigenicity of HEP (approximately 180 chick embryo passages) chick embryo vaccine in cattle was conducted in 1953 in Georgia. (5) It was demonstrated in this study that the vaccine would not produce rabies and that a single injection would protect adult cattle for a period of at least eight months. However, five of eight challenged
calves inoculated at six to nine months of age did not resist challenge indicating that a single injection would not give adequate protection to immature animals.

As rabies decreased there was a corresponding increase in apathy toward the disease. Annual inoculation of dogs came generally to be deemed a nuisance and could not be enforced. It appeared that the continued use of a vaccine with only one year immunity duration would be inadequate to maintain a satisfactory immune barrier. When it was demonstrated that living attenuated virus antirabic vaccine was safe, free of the paralytic factor, and would confer a satisfactory immunity for more than one year, a new approach to the problem was opened up. The early defects in the vaccine (stabilization) having been corrected and a federal license granted, it was decided to use it in mass dog vaccination projects in infected counties only.

**DOG IMMUNIZATION AND CONTROL**

Many infected counties had no funds to apply to a thorough vaccination program and state aid was desirable. Therefore, with a grant from Governor Talmadge a supply of chick embryo vaccine was purchased for free distribution to trouble counties, particularly those in which little or no progress had been made. By agreement with the county commissioners or board of health, vaccine was furnished free provided that the county employed a veterinarian to administer the vaccine, did not charge the owner over $1.00 per dog, put on a satisfactory publicity program for two weeks prior to the program and arranged for clinics to be held at locations convenient to dog owners. Except in Fulton and DeKalb Counties the program was limited to a two week period. Usually 15–20 clinics were provided per county.

During 1952 such programs were conducted in 21 counties. With three exceptions 60–75 percent of the dog population was immunized at clinics and veterinary hospitals. The programs in two counties were poorly organized and the results were not satisfactory. Fulton County, in which most of Atlanta is located, was reasonably well organized but the results were poor and rabies continued unabated. Fox rabies was prevalent in some of the counties and whenever possible simultaneous wildlife reduction programs were conducted by the United States Fish and Wildlife Service.

In March 1953 threatened legal proceedings resulted in instructions from the Assistant State Attorney General’s office that we could no longer furnish vaccine to the counties if an inoculation fee was collected from the owner. It was ruled in essence that the state could not assist counties financially unless free service was offered directly to the people. Since rabies infection was negligible in the south and south central portion of the state in 1952 (Map No. II) but particularly active in the area of Metropolitan Atlanta, it was decided to concentrate our efforts in these heavily infected areas.

From 1953 through 1956 free mass immunization programs were conducted in 33 counties including Metropolitan Atlanta (Fulton and DeKalb Counties). State assistance in some form was provided in a total of 54 counties in the period 1952 through 1956. Subsequent to this program there was a very abrupt drop in the number of positive dogs which continued through early 1956 (Map III) and rabies decreased in the entire state to a degree which would permit investigation of many
individual cases. If a rabid dog was reported and if a good county-state program had been held previously, either a local veterinarian was encouraged to hold a private clinic in the immediate area of the infection or a free clinic was held. Since speed is essential, it is highly desirable that not over four or five days elapse between determining that the offending animal was rabid and holding the clinic. Two free spot clinics were held in Fulton, four in DeKalb County, four in Henry and one in Spalding County during 1954 and 1955.
As shown in Maps I and II, Metropolitan Atlanta had by far the heaviest infection rate in the entire state. All dog inoculations were given at the veterinary hospitals except in rural areas of Fulton County where clinics were held. Veterinarians were inoculating 14,000 to 16,000 dogs annually in Fulton County, 4,000 to 6,000 in other parts of the state.
5,000 in DeKalb and 800–1000 in Clayton. These were distributed throughout the twelve months of the year. Clinics in the rural areas were usually held in the spring.

In April and May, 1953 free dog inoculation programs were conducted in Fulton and DeKalb Counties with chick embryo vaccine. Under this arrangement the State Department of Public Health furnished technical assistance in organization, a representative of the State Health Department attended every clinic and had charge of the vaccine. The counties paid participating veterinarians $10.00 per hour to administer the vaccine and provided clerical and other help at the clinics. Clinics were scheduled at announced locations, dates and hours throughout the counties. The daily newspapers and the neighborhood and rural papers gave excellent coverage. Educational pamphlets were given to every pupil in the schools. Large red posters were put up with arrows pointing in the direction of the clinic location. The radio stations gave spot announcements daily at 7:00 A.M., 12:00 Noon and 6:00 P.M., advising dog owners to have their dogs inoculated and stating where and when the next clinics would be held. Sound trucks patrolled the nearby streets and communities before and during the clinics asking dog owners to bring their dogs in for free inoculation. Short skits were shown on television. Every known practical publicity measure was employed.

In Fulton County 21,998 dogs were inoculated with chick embryo vaccine at the clinics in April and about 14,000 by private veterinarians at their offices using killed virus vaccine during the remainder of the year. Over 8,000 dogs were inoculated at clinics in DeKalb County and 4,000–5,000 privately. An estimated total of between 45,000 and 50,000 dogs were inoculated in the two counties in 1953 or probably 70–80 percent of the total dog population.

There were 28 positive dogs, two foxes and three cattle in the two counties in 1954 and 13 dogs, one cow and one cat in the spring of 1955. There has been one rabid dog in DeKalb County since June, 1955 and none in Fulton County since August, 1955. There is no question but that the 1953 program was the primary factor in controlling the disease in this area. Even though there were a few post program cases the ratio of susceptibles to immunized animals was too low to maintain an epidemic. With the exception of the spot clinics noted above, rabies immunization has been returned to the private practicing veterinarians.

Clayton, the other county in the Metropolitan area, was averaging eight or nine positive dogs each year. In spite of numerous conferences the county would not enter into a state-county program. Annually veterinarians held clinics in the several small municipalities and the usual response was 800–1,000 dogs immunized or probably 12–15 percent of the dog population.

In June and July of 1956 there were five rabid dogs and a cat in the county and four in immediately adjoining areas of Henry and Spalding Counties. Antirabic treatments were issued for thirteen people who had been bitten or otherwise exposed. A state-supported free dog inoculation program was instituted following an intensive educational program. A total of 2,263 dogs and 127 cats were inoculated at the clinics in Clayton County. Veterinarians had held private clinics throughout the county in June and July with about 800 responding, making a grand total of 3,000–3,500 dogs including those vaccinated in private hospitals or approximately 65–70 percent of the dog population. Two free clinics were held in the immediate
areas where the Henry and Spalding County dogs were known to have traveled. Clayton County maintains a dog pound and warden, who was quite active in picking up dogs and contributed much to the success of the program. This program was completed early in August, 1956 and to date there have been no other positive animals in the area, with the exception of one goat which was presumably bitten by a dog from the epidemic area.

State-county dog inoculation programs have been completed in 54 counties in the period 1952-1956 using chick embryo vaccine exclusively (Map IV). We do
not have complete data but more than 175,000 dogs have been inoculated at state-county clinics during this period. We have not recommended re-inoculation of dogs following use of chick embryo vaccine in county programs, even those conducted in 1952, although we have no objection to it if the owner or veterinarian so desires. However, we state in our educational material that dogs inoculated with chick embryo vaccine do not have to be re-inoculated for four years. This, plus free service, is conducive to owner participation and increases dog inoculation in an area.

Map III shows the incidence of rabies in 1956 by species and counties. Through October 1, 1956 there has been a total of 14 positive dogs and this constitutes nearly the entire total for the state. Nine of these plus a cat and goat were in the Clayton County area. Four of the remaining positive dogs occurred in areas previously free of rabies and local control programs were instituted within one week. The most recent positive dog was in a fox endemic area (Coffee County).

There have been 47 positive foxes to date in 1956. They are confined largely to nine south central and southwest counties. Twelve rabid cattle, three cats, one goat, and four solitary insectivorous bats have been reported. Part of these animals originated in areas that had presumably been free of rabies for several months or, in some cases, several years. It is pure supposition but the occurrence of isolated cases in rabies free areas suggests a possibility that the bat or some unknown vector may be responsible for maintaining the disease. Only time can tell.

Although still present the disease is confined to very limited areas and infects a much reduced number of animals as compared to 1946. There are no epidemics. In fact, while not eradicated, rabies is apparently under control for the first time in many years in Georgia.

The decrease in animal rabies is directly reflected in a corresponding decrease in the relative percent of positive brains to the total examined in all the laboratories in the state. From Graph I one will note that in the years prior to 1946 approximately 45 percent of all brain examinations were positive either by direct brain smear or mouse inoculation. The percent positive has steadily declined since 1946 to 8+ percent in 1956. The data are shown in Graph I for the years 1931–1956.

A corresponding decline is noted in the distribution of antirabic vaccine and human deaths from rabies (Graphs II and III). In 1936 antirabic vaccine was issued for 3250 people, the peak year. This was followed by a gradual decline particularly in 1955 and 1956. To date 180 antirabic treatments have been issued for human prophylaxis in 1956. In the period from 1918 to 1946 there was an average of 3 human deaths annually. Since 1946 there have been four human deaths, or one every two years, none of whom had had either antirabic vaccine or hyperimmune serum because exposure was not known until the disease had developed. Since 1948 hyperimmune serum plus vaccine has been used in 74 human cases with severe face and finger bites by rabid animals or animals in which rabies could not be excluded by the history. No deaths have occurred in this group.

An experimental project with respect to the future use of HEP chick embryo vaccine in human rabies prophylaxis is in progress with Dr. John Fox (6), Tulane University Medical School, as principal investigator. The safety of the HEP vaccine has been established by the inoculation of human volunteers, with intra-
dermal injection as the optimum route. Particular study is being given to determination of the size, number and interval between doses required to produce adequate protection, with particular emphasis on the booster effect in persons who have previously taken one or more series of Pasteur treatments. Twenty-five Georgia veterinarians have voluntarily participated in this study. It is probable that HEP chick embryo vaccine will largely supplant vaccine of brain tissue origin in human prophylaxis within a few years.

WILDLIFE RABIES

Organized control of rabies in wildlife began in 1946 under the direction of one of us (Canup). Since rabies was prevalent in foxes and occasionally other animals, control of the disease in both dogs and wildlife was and still is equally important for the state as a unit.

Obviously the customary methods of control practiced for dogs cannot be employed on wild animals. The only available method for large scale use is to reduce the animal population in infected areas as rapidly as possible by supervised approved methods. The Fish and Wildlife Service is limited to study of the animal population, animal ecology, education, organization on the county level, training
local trappers and general supervision. Farmers and citizens are encouraged to take whatever means are necessary to protect their livestock and their families from possible attack and injury. Ballantine and O'Donoghue (7) reported that thorough dog quarantine and inoculation and reduction of the wildlife population in the area were very effective in Alberta. The methods used to reduce the wildlife population were by shooting, traps, snares and poisoned bait. Strychnine, sodium cyanide and sodium fluoroacetate (1080) were used. While proven effective, we cannot sponsor the use of poison in Georgia. Too, the United States Fish and Wildlife Service does not approve of the use of poisons in predator control in populated areas. This is ironclad in regard to 1080.

Upon receipt of information that rabies existed in a given area the county commissioners were asked to sponsor a wildlife control program. To secure federal assistance an agreement was necessary between the chairman of the county board of roads and revenue, Mr. S. M. Canup, Mr. Larrie C. Whitehead, and the senior author representing the State Department of Public Health. In this agreement it was required that the county employ a county trapper for a period of three months and purchase special fox traps. One of us (Mr. Canup) worked with the county trapper for a week or two teaching him where to look for wildlife, to recognize species by tracks, where and how to set the traps, how to camouflage and bait them. Fox or fox and wild bobcat urine is sprinkled near the trap to attract the animals. Odoriferous material such as cracklings, fish, chicken heads, etc. are used for bait immediately back of the trap.

Traps are set on a farm only on request or permission of the owner. Demonstrations are given to interested landowners on the proper procedures for successful trapping. The county trapper has charge of the traps, issuing them to interested farmers, usually about one dozen per man unit. Each line of traps must be visited daily to destroy and remove any animals. If allowed to remain longer they may chew the leg off and escape. If obviously stray dogs are caught they are destroyed. Owned dogs are released. The traps will not break a dog's leg but if the animal is allowed to remain in it gangrene will develop and a three-legged dog will result.

Each owner to whom traps are issued is required to keep a record of the animals caught and report to the county trapper. Some counties pay a bounty of $2.00 to $3.00 per head. The bounty is paid by the county clerk upon presentation of the scalp and both ears as evidence.

The optimum time for trapping in Georgia is from October to April. Food is not as plentiful during fall, winter and spring and the animals are breeding in the fall and rearing young in the spring. In order of numbers caught, foxes are first, followed by domestic cats, skunk, opossum, raccoon and bobcat.

Wildlife generally follow streams, fences or natural barriers and when animals are very plentiful, distinct runways may be noted. Traps are set about three feet back of a runway or near the entrance to a den. Red foxes usually rear their young in a deep burrow or den. Grey foxes do not use a burrow as a rule but rear their young in the open under a bush, in a weed patch or slab pile. Sawdust and slab piles are common throughout the state and are favorite denning places for foxes. There may be several active dens in one sawdust pile.
In addition to trapping, gassing of dens is encouraged when there is evidence that a vixen is rearing pups. If the den is active the ground entering it is smooth and there are bones, feathers, etc. about the entrance. The Fish and Wildlife Service will furnish counties or individuals with CO₂ cartridges at cost, about nine cents each. A fuse on the cartridge is lit and the cartridge is placed as deep as possible in the den and all openings plugged with soil. Every living animal in the den will be killed. Gas cartridges can be used in sawdust piles if the cartridge is placed deep and the opening plugged tight to exclude air, otherwise the sawdust may ignite. Cartridges are not effective in slab piles or in the open where the gas can escape readily. Den gassing is only effective and used during the young bearing period.

Hunters and landowners, particularly if rabies is prevalent, shoot untold numbers of foxes, domestic cats and bobcats in the fields. Although not sponsored by the Fish and Wildlife Service or the State Department of Health, poisoned bait is used quite extensively. Non-fertile eggs, hamburger, wiener and fish are the most common vehicles employed.

The signed agreement permits wildlife thinning anywhere in the county but this action is confined to the known infected area, frequently only a limited area in a county. The result of a three months thinning program varies from a total of 200–800 foxes and a varying number of domestic cats, bobcats and others, depending on density of population, the cooperation of the participating trappers, resistance of fox hunters and the area covered.

The cost per county is from $1000 to $1,200 for the three month period. Any funds paid as bounties are in addition to the above.

Many counties do not have funds available to support such a program. In order to assist them the State Board of Health purchased traps in 1952 to be loaned to the counties on a matching basis with the proviso that the number of traps loaned by the state must be returned in a clean condition at termination of the agreement. Returned traps could then be loaned to other counties.

For a variety of reasons there has been considerable loss in traps. They may not be collected promptly at the completion of the program in a county, some are stolen and in some instances opponents to wildlife reduction throw them in a river or otherwise destroy them.

In 1955 the State Department of Health through its general county aid funds began paying one-half the salary of the local trappers at the rate of not exceeding $6.50 per actual working day for a period of three months. In order to encourage return of state traps these must be returned by the county before the final payment is made for the trapper's services.

As shown in Map V wildlife control programs have been conducted in roughly the same general area as state-county dog immunization programs. Both services have concentrated their efforts in counties with greatest need. Wherever possible dog and wildlife programs were conducted concurrently. By so doing both programs receive the benefit offered by the preliminary education and publicity material. Much dependence is placed on publicity concerning the number of positive animals, livestock losses, and people bitten and pressure by citizens, particularly cattle owners to insist that remedial action be taken. In many counties local and
outside individual fox hunters have been able to prevent wildlife control programs even though an epidemic was in actual progress. In those cases every effort was made to inoculate the dogs while the wildlife epidemic was burning itself out.

In the period 1945 through 1956 Federal-county wildlife control programs have been conducted in a total of 64 of the 159 counties in the state. In a number of counties either the wildlife population was not reduced adequately to control rabies or the population soon returned to normal and control programs were repeated.

A summary of the total animals by species destroyed, cost to the counties, re-
ported livestock losses and their estimated value is shown in Table I. These figures do not represent a total for the entire state but only those reported at the termination of federal-county organized programs.

When indicated, wildlife reduction programs were conducted on the several military reservations in Georgia by the Post Veterinarian, assisted by the junior author (Canup). Fort Benning (8) experienced an outbreak of fox rabies in 1954 with a total of six rabid foxes and one bobcat between August 23, 1954 and January 9, 1955. A reduction program during the period August, 1954 and March, 1956 resulted in a total catch of 257 fox, 360 bobcat, 627 skunk, 194 raccoon, 104 opossum and 55 other species. There have been no rabid animals reported at Fort Benning since January, 1955. This report shows quite dramatically the relative density of the wildlife population in Georgia and the effect of a thorough reduction program.

Critical evaluation of the effects of wildlife control in rabies infected areas indicates that it has been a decisive factor in the reduction of rabies. The data noted in Table I speak clearly in themselves but are even more impressive when evaluated against the variety of local conditions, adverse as well as favorable, which were encountered.

In August, 1955 the United States Public Health Service assigned a veterinarian (Watson) to the Georgia State Department of Public Health on rabies control under the supervision of the senior author (Starr). At the same time the Georgia State Game and Fish Commission assigned a game warden (Jernigan) to wildlife rabies control under the supervision of the junior author and the United States Fish and Wildlife Service. Close liaison by conferences, telephone and correspondence with the central office in Atlanta was maintained. With the report of a rabid animal, regardless of species, a form letter giving the date and species of animal was sent to all county officials, local board of health, county health officer and veterinarians. Depending on the species of animal and circumstances one of us investigated the case and made appropriate recommendations.

By close attention and prompt action in all cases of dog rabies the disease has been reduced to an almost negligible factor by the fall of 1956. There have been
only 14 reports of dog rabies in the entire state during the year through September. Nine of the 14 were in the Clayton County area in July and August immediately prior to the free county-wide mass inoculation programs referred to above. The others occurred as individual cases in widely scattered areas. One occurred in Coffee County which is a known infected area in which a wildlife reduction program is in progress.

Wildlife rabies is confined almost entirely to foxes but as shown in Table I there was a decline of incidence corresponding to that observed in dogs. At the present time (October, 1956) there are two fox endemic areas, one in south central and one in southwest Georgia, but no epidemic.

**DISCUSSION**

Rabies has been a major disease menace to man and animals for many years in Georgia. A rabies control act was made into law in 1945 which provided for compulsory annual inoculation of dogs and methods of control. Control measures according to provisions of the act were inaugurated in 1946. At this time the disease was distributed throughout the entire state involving dogs, foxes, cats, bobcats, cattle, horses, mules and occasionally skunk, raccoon and man. A total of 808 animals were reported positive by laboratory examination with four human deaths.

An intensive drive was instituted to inoculate as many dogs as possible in every county using brain tissue killed virus vaccine. Not less than 250,000 of the estimated 600,000 dogs in the state were inoculated annually for the next three years. The disease began a gradual decline, particularly in the southern two-thirds of the state, and with the decline a decrease in the number of dogs inoculated. Rabies remained quite constant in the general Atlanta and Chattahoochee river area bordering Alabama.

With localization of the disease to about forty counties further reduction appeared possible with state assistance to the counties and the use of living attenuated antirabic vaccine of chick embryo origin. This policy was instituted in 1952 and has continued to date. In 1952 a total of 21 counties conducted state-supported mass inoculation of dogs at a charge of $1.00 per dog. In the period 1953–1956 free mass inoculation of dogs was conducted in 33 counties in which rabies was a major problem. A total of approximately 175,000 dogs were inoculated with chick embryo vaccine in the 54 counties from 1952 to date at state-county clinics.

The incidence of rabies both as to distribution and number of cases has decreased until this date (October, 1956), the disease appears to be under control although not eradicated. In contrast to a total of 808 positive animals in 1946 there is a total of 81 through September, 1956. Fulton and DeKalb Counties with more positive dogs in 1953 than the entire remaining counties in the State have been with the exception of one rabid dog and two bats free of rabies for 14 months. There have been 14 rabid dogs in the entire state, nine of which occurred in June and July in the Clayton County area.

It is of particular interest that the counties in which good mass inoculation programs were conducted even in 1952 have remained either entirely free or almost free of rabies. These counties were all in rabies endemic or epidemic areas. Ob-
ervation indicates that with a good immune barrier in the dog population, rabies in wildlife will exhaust itself in a year or two. This can be hastened by a good wildlife reduction program.

The Georgia Department of Public Health has advocated and used chick embryo vaccine exclusively since 1952 when it was given a permanent license. It is safe, free of serious reactions and confers a solid immunity for a period of at least four years. We have had only two proven cases of rabies in at least 175,000 dogs which is as good as can be expected of any product.

The virus must be alive at the time of injection. This means that the vaccine must be kept under refrigeration at all times. Whenever possible we have personally delivered the vaccine to the participating veterinarian in portable refrigerators. If shipped by common carrier it must be packed with a liberal quantity of dry ice. It should not be allowed to remain in the post office or depot over a week-end particularly in the summer. It must be kept out of direct sunlight at clinics and used within one-half hour after reconstituting. It must be injected into the gluteal muscles, never subcutaneously.

It must be emphasized that if abused or not injected properly the vaccine is of little or no value. Therefore in justice to our patients, the dog, control and administration of chick embryo antirabic vaccine should be confined to graduate veterinarians. It is doubtful that non-professional personnel could use it properly unless given careful orientation and specific training.

Chick embryo vaccine is a satisfactory immunization agent for cattle and horses, however, only HEP vaccine should be used. The LEP product used in dogs may be too virulent for use in these animals.

Our observations indicate that if one could maintain immunization with chick embryo vaccine of 50-60 percent of the dogs under one year of age it would effectively control the disease in these animals. Except for household pets the average lifetime of dogs is about four to five years or the presently recognized duration of immunity conferred by a single injection of chick embryo vaccine.

Where rabies exists in wildlife prompt reduction of this population is of great value. The disease itself kills many animals, and in areas with a severe epidemic, may almost completely decimate the fox population. It has been demonstrated in many counties that organized reduction programs as practiced in Georgia hasten the work of nature but will leave an adequate population, particularly foxes, to maintain the balance of nature and protect, even improve, fox hunting for the devotees of that sport.

Probably the greatest handicaps that we have encountered are twofold:

A. Our inability to convince the fox hunters that we are not attempting to eradicate the fox and thus destroy their major sporting activities. The hungry and stupid foxes are caught. The remainder have more food and are too smart to become involved with obviously artificial situations.

B. Compliance with our recommendations concerning rabies control in both dogs and wildlife is entirely voluntary with the result that in many instances we must wait until the disease attains epidemic proportions before corrective action can be taken. Instead of working ahead of the disease we are behind it.
SUMMARY

Except for sporadic annual dog immunization clinics in populous centers and in veterinary hospitals, rabies in Georgia prior to 1945 followed the dictates and limitations of nature. With passage of a Rabies Control Act in 1945 organized control programs were instituted by the State Department of Public Health and the United States Fish and Wildlife Service working in collaboration.

Because of the widespread infection in the state, immunization of dogs and wildlife control were encouraged throughout the state, with reduction and concentration of the infection to about 40 counties in 1952 efforts were concentrated in known infected areas.

Chick embryo vaccine was adopted as the vaccine of choice by the State Health Department in 1952 and used exclusively thereafter in state-county mass inoculation programs. A period of at least four years immunity has been postulated and although it is not discouraged we do not require annual inoculation. A total of at least 175,000 dogs have been inoculated with this product with no serious reactions. There have been two proven rabid dogs among those previously inoculated with chick embryo vaccine since 1952. The use of chick embryo vaccine has been a major factor in the reduction of rabies in the counties in which mass inoculation programs were conducted. The availability of this product poses a problem to the profession which must be solved in the very near future.

State-country programs have been conducted in 54 of our 159 counties beginning in 1952. The number of rabid animals in 1956 is the lowest in the history of the State Health Department. Up to October 1 there had been 14 rabid dogs, 47 fox, 12 cattle, three cats, one goat and four bats.

Wildlife control programs have been conducted in 64 counties since 1945. In some counties it has been necessary to repeat programs due to over-population with wildlife, particularly foxes, or recurrence of the infection. During this period 22,055 foxes, 2,144 domestic cats, 1,969 skunks, 1,793 opossums, 1,198 raccoons, 575 bobcats and 6,245 stray dogs or a total of 45,939 animals were destroyed in official reduction programs exclusively in known infected areas. Wherever possible dog inoculation and wildlife programs were conducted concurrently.

The significance of insectivorous bats as a possible reservoir for the maintenance and transmission of rabies virus to other species of animals is unknown at present. This field of rabies rates further study.

Rabies in dogs and wildlife is apparently eradicable. To achieve this, however, authority, personnel and funds must be made available on the state level to take whatever measures are necessary to confine and control the infection promptly wherever it may exist. In any state where the disease is present in dogs and wildlife the state health department and wildlife control departments should work in complete coordination.

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REPORT OF COMMITTEE ON RABIES

V. D. CHADWICK, Jackson, Mississippi, Chairman; E. S. TIERKEL, Atlanta, Georgia; J. B. HENDERSON, Fort Worth, Texas; DAN HALEY, Albany, New York; H. A. MILO, Harrisburg, Pennsylvania; I. M. MULTHROP, Salisbury, Maryland; JOHN I. CURTIS, Salt Lake City, Utah

CURRENT TRENDS IN RABIES MORBIDITY

The annual incidence of rabies in the United States continued to decrease during calendar year 1955 according to the weekly reports from the United States Public Health Service and the annual survey by the United States Department of Agriculture. According to the latter report there were 5,844 total cases in 1955; 2,657 in dogs; 343 in cats, 956 in livestock; 1,223 in foxes; 586 in skunks and 48 in other species of wild animals. This represents a drop of 1,438 total cases from the 1954 incidence. Success in canine rabies control programs is reflected by almost a 50 percent drop in the number of dog cases from 1954 to 1955. At the same time the number of cases reported in wild animals continues to rise, particularly in foxes and skunks.

One of the biggest reductions in incidence from the previous year occurred in Texas; this drop was due mainly to the highly successful control program in Houston-Harris County which in 1954, contributed more than half the State total. A slight drop was noted in skunk rabies in the Missouri Valley States and in dog rabies in the Midwest between the Great Lakes and the Ohio River. There also has been a decrease in total rabies cases in the Southeast. Two states which experienced serious rises in incidence during 1955 were California (skunks in the northern counties and dogs in Los Angeles County) and Pennsylvania (principally foxes in southeastern counties). Five human deaths were reported during 1955 and the same number have been reported thus far in the first six months of 1956. It is interesting to note that 20 percent of the human cases in the United States during the past four years have been attributed to wild animal exposures.

BAT RABIES

Approximately 170 isolations of rabies virus from bats have been made since June 1953. These have been reported from Florida, Pennsylvania, Texas, California, Montana, Ohio, Louisiana, New Mexico, Georgia, Utah, Alabama, Oklahoma, Minnesota and New York. Four species of solitary or tree-living bats and eight species of colonial or cave-dwelling bats have thus far been implicated. The Mexican free tailed bat in southwestern United States leads in the frequency of isolations. Florida State Board of Health has recently reported rabies found in suckling bats found with the mother bat in Spanish moss tree roosts.

Twelve of the total number of positive diagnoses in the country have been associated with episodes involving the biting of human beings. One human rabies death which occurred in October 1951 was investigated retrospectively in Texas
**TABLE 1**

*Incidence of Rabies in the United States Since 1838*

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cattle</th>
<th>Horses</th>
<th>Sheep</th>
<th>Swine</th>
<th>Cats</th>
<th>Goats</th>
<th>Misc.</th>
<th>Man</th>
<th>Total</th>
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<td>413</td>
<td>32</td>
<td>164</td>
<td>42</td>
<td>207</td>
<td>11</td>
<td>44</td>
<td>47</td>
<td>9,412</td>
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<tr>
<td>1939</td>
<td>7,386</td>
<td>358</td>
<td>36</td>
<td>17</td>
<td>38</td>
<td>369</td>
<td>10</td>
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**Totals**: 116,141 (11,628) 590 697 884 6,857 160 15,081 434 152,472

**MAP I**

*Rabies Reported in 1955*

*Total Cases Reported - 5946*

U.S. Department of Agriculture
### TABLE 2

Rabies in the United States by States during the Year 1955

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Alaska reports 3 cases in dogs and 2 in foxes.
Hawaii reports that rabies has never occurred in the Territory.
Puerto Rico reports cases of rabies in 4 dogs, 3 cattle, 2 horses, 1 swine, and 16 mongooses.
### Table 3

**Distribution of Rabies by States for the Period 1846-1866, Inclusive**

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and attributed on epidemiological evidence to the bite of a bat. A second human rabies death occurred in Texas in January 1956 in a State Health Department scientist who had been working in the field on the bat rabies project there. In spite of the patient's close association with infected bats, no history of bat bite could be elicited.

The Communicable Disease Center has begun a thorough epizootiological investigation of bat rabies and associated ecological factors in the bat colony at Carlsbad Caverns, New Mexico.

Your Committee on Rabies recommends:

1. Since rabies is such a public health problem, as well as an economic factor in the production of livestock, and with the knowledge we have that will work in the control of rabies; adequate nation-wide program should be inaugurated to control this disease with eventual eradication in mind.

2. Cooperation of all rabies control authorities with various departments of wildlife service for better control activities of wild animal rabies since this is the source of our infection in many areas.

3. Recognize the dog as a domestic animal whereby all State and Federal departments can fully cooperate for complete eradication.

4. Effective programs of local rabies control including mass canine anti-rabies immunization of dogs and elimination of strays by the operation of local pounds or humane shelters where stray dogs may be kept for a few days until claimed, or used for intelligent humane teaching or research projects, or otherwise humanely disposed of.

### TABLE 3—Continued

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TWELVE YEARS' SUCCESSFUL VACCINATION OF FARM HERDS WITH CRYSTAL VIOLET-GLYCEROL HOG CHOLERA VACCINE


Ames, Iowa, and Live Oak, Florida

The immunization of hogs against hog cholera with vaccine was attempted before the discovery of the simultaneous serum and virus method (1). Many different chemicals and methods were employed to produce vaccines. Glycerine was one of the first substances used with virus blood as a vaccine, but this method of production did not prove practical (2). Later crystal violet was employed in making a vaccine (3). Extensive experimentation proved this vaccine would give 96 to 98 percent protection against hog cholera virus three weeks after vaccination (4). Crystal violet did not control the contamination in all lots of vaccine; so other substances were combined with it for this purpose. Disodium phosphate was used for several years, and by the end of the fiscal year June 30, 1942, 262 farm herds, consisting of more than 14,000 head of pigs, had been vaccinated (5). Tests for immunity of pigs from many of these herds at time of marketing showed that 83 percent were satisfactorily protected against hog cholera (6). Disodium phosphate as an added preservative was not entirely satisfactory because too many lots of vaccine were not rendered sterile (7). Continued search was made for a better preservative agent. As a result of this search, crystal violet-glycerol (CVG) vaccine was developed by Dr. Frank Tilley of the United States Department of Agriculture (8). Doctor Tilley obtained a patent, No. 2369267, dated February 13, 1943, on this method of producing vaccine and assigned it to the Secretary of Agriculture and his successors in office. Extensive experimentation had shown that 93 percent or more of the pigs vaccinated with crystal violet-glycerol vaccine were protected against hog cholera (9). Vaccination of farm herds was begun in 1943, and results of this experiment are presented in this paper.

MATERIAL

The pigs used in the preparation of vaccine were purchased from farms in an area where hog cholera was not present and vaccination against hog cholera was not practiced. These pigs were brought to the experiment station at Ames, Iowa, and two pigs were tested for susceptibility to hog cholera. If they proved susceptible, a sufficient number of pigs to make the required amount of vaccine were injected with a virus which had been previously used to make a satisfactory vaccine. On the seventh day after injection with virus the pigs were bled from the anterior

* Retired, Ames, Iowa.
vena cava under aseptic conditions. Previous experiments had shown that the virus reaches its maximum growth in the pig between the sixth and eighth day after injection (10). The blood was defibrinated mechanically at the time of bleeding and passed through sterile gauze to remove the fibrin. It was then cultured on blood agar plates for bacterial contamination. A solution of glycerine and crystal violet was prepared by adding one part of crystal violet to 400 parts of glycerine (five gm. crystal violet to 2,000 cc. of glycerine) and heating it in a steam sterilizer for 30 minutes. This solution was then added slowly to the blood while being agitated in the proportion of one part of crystal violet-glycerol to four parts of defibrinated blood. The solution and blood were thoroughly mixed, and the mixture was incubated at 37.5°C. for 14 days. The vaccine was shaken vigorously once each day during the period of incubation. Experiments proved that vaccines could be made at room temperature, but incubating at 37.5°C. hastened the process and made a vaccine which gave a more uniform reaction. When completed, the vaccine should be a dark purplish-red color and free from solid particles, sediment, and bacterial growth.

There were 30 lots of CVG vaccine used in the 12-year period. In some years as many as five lots of vaccine were used; in other years only one lot of vaccine was used; and in three different years the same lot of vaccine was used.

Each lot of vaccine was tested for potency before use by injecting pigs with doses of 1 cc., 2.5 cc., 5 cc., and 10 cc., two pigs being used for each dose. After 21 days the eight pigs were challenged with virulent hog cholera virus. Simultaneously, equal dosages of a previously tested vaccine were injected into pigs from the same lot for comparison.

The potency of the vaccine was considered adequate if the pigs injected with 2.5 cc., 5 cc., or 10 cc. withstood a 2 cc. challenge dose of virus. The pigs injected with 1 cc. of vaccine may show a reaction or in some cases die.

**METHOD**

In carrying out farm experiments with crystal violet-glycerol vaccine, we made no solicitations. The owners, however, made requests for the vaccine to be used on their pigs, at which time they were given all available information regarding the advantages and disadvantages of the vaccine. Farms were accepted in communities where no hog cholera existed. Cholera infected or exposed herds were carefully avoided. Most of the farms were located within a 10-mile radius of the Hog Cholera Research Station, Ames, Iowa. Small herds, consisting of less than 100 head of hogs, were usually selected in order to distribute the vaccinated herds over as much territory as possible. Because of limited funds and personnel, only a small number of herds could be vaccinated each year. Therefore, most farmers were kept on the program for from two to four years and then dropped so that new herds could be taken on. This procedure continuously established the vaccination of additional herds in new areas. Four farmers have been kept on the program for the entire 12-year period. Larger herds have been vaccinated in recent years because of changing farm conditions and because more large herds are raised. Two herds of pigs were vaccinated on some farms in a year—the spring herd and the fall herd.
When requesting the use of the vaccine, which was free of charge, the herd owner was asked to sign a statement agreeing to deliver to the Hog Cholera Research Station at Ames, Iowa, whatever number of hogs were required for an immunity test of his herd. The price paid for these hogs by the Government was the prevailing market price at the time of delivery. As soon as the pigs had been weaned for one to two weeks, they were vaccinated by injecting five cc. of vaccine subcutaneously into each pig under 75 pounds and 10 cc. into each pig over 75 pounds.

When the pigs were ready for market, two or more pigs were selected for the immunity test. They were delivered to the experiment station, and two cc. of a virulent hog cholera virus was injected subcutaneously into each of the test pigs. Daily observations were made, and the condition of the pig was recorded by a point system; if the pig remained normal, no points were marked against him. If he did not eat a normal daily ration and was somewhat slow in eating, one point was marked against him; if the pig ate only a small amount of feed and soon went back to the nest, two points were given to him; and if he refused to eat and stayed in the nest, three points were given. At the end of a 10 to 14 day period, all pigs with no points were marked “normal (N)”; those with one to 10 points were marked “slight reaction (SI)”; and those with over 10 points were marked “severe reaction (Sv).”

RESULTS

The results obtained in each of the 12 years cannot be discussed separately; so only the differences will be pointed out.

Crystal violet-phosphate (CVF) vaccine had been used for several years with good results before 1943. When crystal violet-glycerol (CVG) vaccine was first used on farm herds in 1943, half of each was vaccinated with CVG and half was vaccinated with CVF for comparison. This division of herds for comparison accounts for the small number of hogs vaccinated with CVG in 1943. Since crystal violet-glycerol vaccine gave the best results, it was used to vaccinate all herds in following years.

More than 20,000 head of hogs were vaccinated with CVG vaccine, but pigs could not be challenged from all the herds because of a shortage of funds and facilities. This shortage also accounts for the grouping together of the records for the years 1950 and 1952. Some herds of pigs were vaccinated in 1951, but no animals were obtained for challenging in that year. Only the herds from which pigs were tested for immunity are included in this report. Table 1 is a summary of the vaccination experiment from 1943 to 1955. Tests for immunity were made on 523 head of hogs from 42 farms representing 12,167 head of pigs vaccinated.

The age at which pigs were vaccinated ranged from two to 26 weeks. The average age at which pigs were vaccinated in 1943 was 12 weeks. This age has decreased during the 12-year period until the average age in 1955 was 9 weeks. This decrease in age in due to the earlier weaning age in recent years. The vaccination of pigs before weaning accounted for a six weeks' average vaccination age in 1953.

The weight of the animals at the time of challenging varied from 70 to 360 pounds. The average weight was 230 pounds.
**TABLE I**

*Summary of Twelve-year Record of Vaccination With Crystal Violet-Glycerol Vaccine*

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* N—normal; Sl—slight; Sv—severe; D—died.
The period of time between vaccination and challenging varied from 90 to 338 days. In 1943 the average number of days was 188, whereas that in 1955 was 133. The lower average in 1955 can be attributed to the earlier age, in recent years, at which hogs are ready for market.

The reactions of the pigs after challenge are recorded in the table as normal (N), slight (Sl), severe (Sv) or died (D). Pigs which showed no reaction were held for 14 days before being released as normal. Pigs which became sick after challenging showed the first symptoms on the fourth or fifth day in most cases. A few pigs were sick on the third day. The first symptoms were slight depression of the appetite and less vigorous consumption of the feed. On the second day of sickness the pigs may eat a small amount of feed or refuse to eat at all. The pigs which were recorded as having a slight reaction were not entirely off feed more than two days and were eating a full ration by the sixth day after the first symptoms of sickness appeared. Loss of weight was very slight during this time. The pigs marked "severe reaction" showed varying degrees of symptoms. Some had a mild reaction for a number of days so that the points would be more than 10. Others would be off feed for as long as 30 days and would then recover. The percentage of pigs showing each reaction is recorded in the table for each year under the headings "normal," "slight," "severe," or "died."

A comparison of the percentage of protection for each year shows that this varied greatly in different years. Even when the same vaccine was used in two different years, the results were different. This variation of protection also varied in different herds. In some years all of the pigs tested, or 100 percent, were adequately protected. In other years, as in 1954, about half the pigs were adequately protected. However, the survival rate was high—82.8 to 87.5 percent.

Those pigs which had no reaction or only a slight reaction were considered adequately protected. There were two years when this protection was 50 percent or less. The average for the 12 years was 77.5 percent.

The death loss after challenge was relatively small for each year and in three years there were no deaths. The lowest survival percent was 82.8.

The pigs on four farms were vaccinated each year during the 12 years. Representative pigs were challenged from one of these farms each year. None of the pigs challenged in 11 of the years had any reactions. The pigs challenged in the other year (1952) had severe reactions, and one pig died. Pigs were not challenged from the other three farms consistently, and the results were more variable. The challenging of representative pigs from all herds of pigs vaccinated since 1953 indicates that there are some herds which will not develop immunity, some in which immunity lasts for only a short time, and others in which the immunity lasts for a longer time.

In 1953 new herds were added to the vaccination program. In most of these herds the sows had been vaccinated with serum and virus. The pigs were vaccinated before weaning to test the feasibility of this time of vaccination. Two pigs from each herd were challenged, giving a total of 45 head. Six of the challenged pigs died. Five of them were from different farms, and all were from sows which had been vaccinated with serum and virus. The challenged mate in each case had a severe reaction. This would indicate that pigs from serum-virus-treated sows do
not develop a strong immunity when CVG vaccine is used before the pigs are weaned.

In addition to the farm vaccinations carried on at the experiment station at Ames, Iowa, cooperative vaccination was conducted with veterinarians in eight different States during an eight-year period (11). Vaccine was supplied free to practicing veterinarians who were required to supervise and report results of all herds treated. Reports were received from 175 veterinarians on 1,974 herds consisting of 49,950 pigs treated. No losses were reported in 164 of the 175 reports. Most of the losses reported occurred during the first two years when the vaccine was distributed. Some reports stated that pigs remained normal when cholera was present in neighboring herds and in some cases when cholera appeared in unvaccinated pigs on the same premises.

**DISCUSSION**

The challenging of representative pigs from only some of the herds vaccinated with CVG vaccine may have left doubt as to whether all herds were adequately protected. However, the location of these herds in scattered areas gave ample opportunity for them to be exposed naturally. In some instances vaccinated herds were located where there were outbreaks of cholera on adjoining farms.

Hog cholera did not occur in any of the vaccinated herds, nor was there sickness in any herd immediately after vaccination. Persons doing the vaccination were actively engaged in research on hog cholera and doing the postmortem work at the Hog Cholera Research Station. Proper disinfection and sterilization of materials taken to the farms when vaccinating prevented the spread of hog cholera virus.

Sickness occurred in some herds at various lengths of time after vaccination. The herds were inspected immediately and postmortem examinations made. Blood and tissue suspensions were injected into susceptible pigs, and bacteriological cultures were made. None of these cases was proved to be hog cholera. Swine erysipelas, pasteurellosis, or salmonellosis were diagnosed in most cases. Heavy parasitism was found to be the cause of losses in some herds.

Experimental evidence is lacking for an explanation at this time as to why some herds are adequately protected with CVG vaccine and other herds are not when the same lot of vaccine is used. If one can judge by the death losses which occur after the use of all other hog cholera vaccines on the market today, the same immunizing failures exist when they are used. Hog cholera virus has been demonstrated in pigs which have become sick after the use of all the hog cholera immunizing agents on the market at this time (12).

**SUMMARY**

The search for a vaccine which will protect pigs against hog cholera virus but will not cause the spread of the virus has lead to the study of crystal violet-glycerol vaccine under farm conditions.

More than 12,000 head of pigs on 42 widely scattered farms were vaccinated in a 12-year period, and representative test pigs were challenged with virulent hog cholera virus at marketing age. Hog cholera did not appear on any of these farms
during this period. Of 523 head of representative pigs tested, 482, or 92.8 percent, survived a challenge dose of virus. Slight or no reaction was observed in 77.5 percent.

REFERENCES

REPORT OF THE COMMITTEE ON NATIONWIDE ERADICATION OF HOG CHOLERA


The Committee on Nationwide Eradication of Hog Cholera has held meetings both here and in Washington, D. C., during the past year. The meetings have been exceptionally well attended, and very keen interest has been shown in the possibilities of beginning an all-out eradication program.

Results accomplished since the first committee report in 1951 have seemed slow, yet when these results are studied in aggregate, it can be seen that much of the foundation has been laid for a program leading to the eradication of hog cholera. Thanks to vesicular exanthema, more than 90 percent of all garbage that is now fed to swine is cooked before feeding. Eight states have outlawed the use of virulent virus as an immunizing agent. Educational programs have been launched in other states, so that now less and less fully virulent virus is used within these states even though it is permitted. Successful field tests have been conducted to prove the benefits of non-virulent vaccines. You have just heard the report of Doctors Torrey and Zinober of Ames, Iowa, concerning 12 years successful vaccination of farm herds with Crystal Violet vaccine. The work being done in Dale County, Alabama seems to confirm the results of this report. Through the continued efforts of this Committee, the Florida Livestock Board in cooperation with the Agricultural Research Service has established a pilot test area in Suwannee and Hamilton Counties, Florida, to study methods not utilizing fully virulent virus and to observe and apply other accepted control practices. There is much left to be done, however, to assure the success of an eradication program.

Your Committee wishes to reaffirm the recommendations of the Committee in the past and requests this organization to take the lead in beginning an all-out eradication program. Close contact should be maintained between the United States Livestock Sanitary Association and all groups interested in the production of swine, the control of diseases of swine and research pertaining to swine disease in order that all information may be made available to the industry at the earliest possible date. We recommend that the information pertaining to hog cholera gathered by a committee of the United States Livestock Sanitary Association be brought up to date and published as soon as possible in pamphlet form, just as the pamphlet pertaining to Brucellosis is published. Other organizations should be encouraged to publish all the available information they have on this disease.

We realize the need for additional research. The Agricultural Research Service, state Livestock Sanitary officials, agricultural colleges, and all research institutions
should be encouraged to lend their efforts to a further study concerning hog cholera, methods of its control, its immunology and all factors related to its control.

We wish to re-emphasize our belief that the eradication of hog cholera is possible—but not so long as mass production and field use of fully virulent hog cholera virus is permitted. Immunizing agents other than fully virulent virus have been approved and accepted by a majority of Agricultural Research Service workers, representatives of the swine industry, practicing veterinarians, and Livestock Sanitary officials, as evidenced by the fact that 72 percent of all hogs vaccinated in 1956 were injected with such products while only 28% were inoculated with fully virulent virus and serum.

Your committee wishes to express its belief that the time has arrived when a hog cholera eradication program should be begun and that there is no longer a need for fully virulent virus in the control of the disease. Therefore, we request that the following action be taken:

1. (A) The United States Livestock Sanitary Association recommend that a hog cholera eradication section be immediately established in the Animal Disease Eradication Branch of the Agricultural Research Service and that funds be made available from the present budget to the extent possible, for the operation of this section.

   (B) The United States Livestock Sanitary Association further recommend through its Advisory Committee to the Agricultural Research Service that budget allowances be made in the future for the conduct of a complete and effective hog cholera control and eradication program.

2. The United States Livestock Sanitary Association recommend to the United States Department of Agriculture that the production for sale and distribution of fully virulent hog cholera virus for field use shall be banned not later than January 1, 1958, or as near that date as essential legislation will permit.

3. The United States Livestock Sanitary Association recommend to the United States Department of Agriculture that the use of fully virulent hog cholera virus at public stockyards be discontinued at the earliest possible date.

The responsibility of inaugurating a hog cholera eradication program, as had been true of other disease eradication programs in the past, must rest with the Federal Government with the cooperation of the individual states. The desire to eradicate rather than to live with the disease must become instilled in the swine producers. State and Federal Livestock Sanitary officials are urged to take the responsibility of seeing that all interested farm groups are made aware of the tremendous loss from the disease and what can be expected from the use of modern methods of control. Sound programs of control and eradication must be inaugurated within the states if federal aid is to be expected. The committee recommends that the following measures be taken by all states:

1. Discontinue the use of fully virulent hog cholera virus as immunizing agent against hog cholera of swine moving from all places of public sale.

2. Promote legislation or regulations prohibiting the field use and sale of fully virulent hog cholera virus in their states.
3. Heat-treat all garbage used as swine feed to prevent the introduction and spread of the disease through this medium.

4. All states include hog cholera as a reportable disease.

5. Encourage laboratory confirmation of reported cases of hog cholera.

6. Prohibit movement of swine from premises where diagnosis has been made until reasonable danger of spread no longer exists.

7. Properly dispose of all carcasses dead of the disease.

8. Prohibit movement of swine that had been immunized with fully virulent live hog cholera virus for a period of twenty-one days after inoculation.

9. Clean and disinfect all facilities used in the handling of hogs infected with or exposed to hog cholera.

10. Develop and distribute informative pamphlets and fact sheets on the affects of hog cholera to the swine industry, and thereby indoctrinate practitioners, county agents and farm representatives on the need for control and eradication of the disease.
ATROPHIC RHINITIS. VIII. THE ALBINO RAT AS AN EXPERIMENTAL CARRIER


Beltsville, Maryland

INTRODUCTION

A few months after the discovery of atrophic rhinitis in the swine herd of the Animal and Poultry Husbandry Research Branch, at the Agricultural Research Center, Beltsville, Maryland, in April 1952 (1), characteristic lesions of the disease were observed in a number of weanling pigs which, to our knowledge, had not come in direct contact with affected animals. At about the same time several cats frequenting the barns in which the pigs in question had been farrowed were observed to be suffering from a respiratory disorder. These two observations led one of us (LAS) to consider the possibility that mammals other than swine, wild birds, and insects that invade premises occupied by swine harboring the etiologic agent(s) of atrophic rhinitis might perhaps in some way become carriers of the organism(s) and, therefore, serve to disseminate them from infected to clean premises. In view of this possibility, it was considered that if the causative agent(s) of atrophic rhinitis could be shown experimentally to remain infective for an appreciable period in a non-porcine animal of the kinds just mentioned, the occurrence of the disease in the weanling pigs in question could perhaps be accounted for and further information might be obtained as to the nature of the infective agent(s).

Since rodents had originally been considered as possible carriers of atrophic rhinitis, an investigation was initiated to determine whether rats could serve as carriers of the infective agent(s) of the disease. Laboratory-raised albino rats were used in this study. This work was done during the period from October 1953 through June 1956 at the Agricultural Research Center, Beltsville, Maryland.

EXPERIMENTAL PROCEDURES

Eleven experiments, each involving from two to 20 albino rats and a single litter of young pigs, were carried out. Some of the rats and some of the pigs involved in each experiment served as test animals; others as controls. Facts relative to the sources of infective material and the methods of its preparation and administration, the origin of the experimental animals, the conditions under which they were maintained, and other pertinent information which are generally applicable to all of the experiments are summarized below. Other details which are not generally applicable are, for the convenience of the reader, included in the description of the experiment to which they pertain.

Preparation of Infective Material

The infective material used in these experiments was taken from pigs which were part of a herd naturally infected with atrophic rhinitis. Some of the pigs had developed atrophy of the turbinate bones, whereas others had normal turbinates. These pigs were killed, and the snouts were removed by making a transverse cut at a point slightly anterior to the inner canthus of the eye. The snouts were then cut longitudinally through the septum, which was removed, thus exposing the entire nasal mucosa. The mucosa was stripped from the bone by means of a sterile scalpel, suspended in a small amount of physiological saline, and macerated, using a mortar and pestle. The resulting suspension was then filtered through surgical gauze to remove the large particles of host tissue, and the filtrate stored in 10-ml. serum bottles. This material was instilled into the nasal cavity of the rats while still fresh or after thawing at room temperature following storage in a dry-ice chest. In both instances the infectious material was held at 4°C. between instillations. Because of the association of a protozoan, *Trichomonas* sp., with lesions of atrophic rhinitis reported by Switzer (2), Spindler, Shorb, and Hill (3), and other workers, this material was examined for these organisms by direct microscopy and by culture methods before it was instilled into the rats. Although the concentration of the infective agent(s) in the suspensions was not known, in the later experiments an attempt was made to produce relatively uniform doses by mixing one part of nasal scrapings and two and one-half parts of diluent by weight at the time the suspensions were made.

Handling of Experimental Animals

Laboratory-raised albino rats of both sexes, about one-quarter grown, were used in these tests. The rats were anesthetized by ether inhalation, their noses were washed out with physiological saline; and the washings examined for *Trichomonas* sp. by direct microscopy. Each side of the nose was then instilled with approximately 0.02 to 0.03 ml. of the tissue suspension by means of a small capillary pipette. After instillation the rats were maintained in wire cages until autopsied. Except in experiment 3, a comparable group of uninstilled rats was selected to provide a source of normal rat material for instillation into control pigs.

The pigs used in these experiments were farrowed by sows of the breeding herd maintained at the Animal Parasite Laboratory, Agricultural Research Center, Beltsville, Maryland. The sows had no lesions characteristic of atrophic rhinitis which were visible by means of the rhinoscope (4), (5) and the pigs were farrowed in pens which had been thoroughly cleaned and disinfected with two percent lye solution prior to farrowing. The experimental pigs had direct contact only with the sow that farrowed them and were usually weaned when they were three to 10 days old and transferred to concrete-floored pens that had just been thoroughly cleaned and disinfected in the same manner as the farrowing pens. These pigs were fed a diet of pelleted pig starter, supplemented during the first two weeks with cow's milk, and were put on experiment when they were three to 27 days old.

Some of the experiments were conducted in outdoor pens having concrete floors, which were elevated about three feet above the ground and were separated by a
space of 15 to 20 feet. Others were carried out in concrete pens, having walls about five feet high, in a building used only for work on atrophic rhinitis. Precautions were taken to prevent the accidental introduction of infective material into the pens housing the pigs by requiring all personnel working on the project to disinfect their boots in a lye bath before entering and on leaving each pen, and to wash and change to clean coveralls before handling the control pigs. Isolation was further improved by destroying mice and flies in the building housing the experimental pigs. Before starting an experiment the pigs were examined by means of a rhinoscope to detect shrinkage of the turbinates. Nasal washings and feces were also examined for Trichomonas sp. by the methods previously mentioned. Except in experiment 3, each litter was divided into three groups to provide a means of determining the effect of instilling nasal scrapings from normal rats into the noses of the pigs.

EXPERIMENTAL FINDINGS

The pertinent data obtained from the five experiments in which atrophic rhinitis was transmitted to pigs by using the rat as an experimental carrier of the causative agent(s) are summarized in Table I. The important features of the data pertaining to each of these experiments are described as follows:

Experiment 1

Twenty rats and eight 17-day-old pigs were employed in this experiment. Ten rats served as controls and received nothing. The remainder received five instillations each of a pooled suspension of scrapings from three mature pigs.

TABLE I

Summary of Data Obtained from Experiments in which Atrophic Rhinitis (AR) was Transmitted, Using the White Rat as an Experimental Carrier

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>No. of Rats</th>
<th>Time in Rats</th>
<th>No. of Pigs Instilled</th>
<th>Age of Pigs</th>
<th>No. of Instillations to Pigs</th>
<th>Pigs Instilled with Infectious Material</th>
<th>Visible Lesions First Observed</th>
<th>Control Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AR+ (No.)</td>
<td>AR- (No.)</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>9-35</td>
<td>3</td>
<td>17</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>20-26</td>
<td>2</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>21</td>
<td>1</td>
<td>26</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>21</td>
<td>4</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>23-30</td>
<td>2</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Total or spread...</td>
<td>2-10</td>
<td>9-35</td>
<td>12</td>
<td>14-26</td>
<td>1-9</td>
<td>8</td>
<td>4</td>
<td>15-45</td>
</tr>
</tbody>
</table>
which had developed atrophy of the turbinates as the result of natural exposure. The infectious nature of this material for pigs was previously demonstrated by direct transfer to susceptible pigs.

The first instillation administered to the rats contained fresh material, the other four were made after the suspension had been stored one, four, five, and six days, respectively, at 4°C.

Beginning nine days after the last instillation and ending 20 days later, one rat from each group was killed every other day on weekdays, except on the last day when two from each group were sacrificed, to provide fresh material for instillation into the noses of six pigs; three receiving the atrophic rhinitis positive material, and three receiving scrapings from the normal rats. Two pigs were used as uninfected controls.

**Results.** One pig developed atrophy of the turbinates, as determined by rhinoscopic examination, 45 days after the first instillation of infective material. The other two pigs in this group were observed to have shrunken turbinates 23 days later. These findings were confirmed when these pigs were killed and examined post mortem 124 days after the first instillation of infective material. All three pigs showed definite shrinkage of the ventral turbinates and deviation of the septum. The control pigs had normal turbinates when examined post mortem on the same day.

**Experiment 2**

Fifteen rats and seven 14-day-old pigs were used in this experiment. Five rats were used as controls and were not instilled. The remainder were instilled as follows: Five rats received one instillation of a suspension of nasal scrapings from a pig which developed atrophy of the turbinates 23 days after instillation with infective material. These rats died within three days from a condition which appeared to be a respiratory infection, and were refrigerated at 4°C. Immediately after the last rat died, the nasal mucosa of all five rats was scraped and the scrapings pooled in physiological saline. After centrifugation, this material was instilled into the noses of five additional rats.

Twenty, 23, 24, 25, and 26 days, respectively, after instillation, one rat from each of the two remaining groups was sacrificed, the nasal mucosa scraped, and the scrapings were suspended in physiological saline. This suspension was centrifuged, and the sediment instilled into the noses of four pigs; two pigs receiving the scrapings containing infective material and two pigs receiving the scrapings from normal pigs. The three remaining pigs were used as uninfected controls.

**Results.** One pig died 36 days after the first instillation of infective material from an unknown cause. Post-mortem examination revealed that the left ventral turbinate was shrunken and the septum was bowed to the right. Atrophy of the ventral turbinate bones was observed in the second pig of this group when it was examined rhinoscopically 69 days after the first instillation of infective material. This diagnosis was confirmed at the post-mortem examination 27 days later when both ventral turbinates were found to be residual. The turbinates of the control pigs did not show lesions typical of atrophic rhinitis when these pigs were ex-
Experiment 3

Two rats and eleven 26-day-old pigs were used in this experiment. The rats received one instillation intranasally with a pooled suspension of nasal scrapings in physiological saline from 12 "normal" pigs from a herd in which atrophic rhinitis was known to be present. Normal rat material was not provided in this experiment because it was not considered necessary to control this phase of the experiment at the time it was carried out.

Twenty-one days after instillation the rats were sacrificed, and their nasal scrapings were pooled, suspended in physiological saline, and instilled into the nose of a 26-day-old pig. Ten litter mates received nothing and were used as uninfected controls.

Results. Forty-one days after instillation, rhinoscopic examination of the instilled pig disclosed a slight but definite shrinkage of the ventral turbinates. Twenty-seven days later this condition was confirmed by post-mortem examination when both ventral turbinates were observed to be residual and the septum deviated to the left. Atrophic rhinitis was not observed in 10 uninstilled littermates during a period varying from 70 days to more than two years after the beginning of the experiment. Nine of these pigs were examined post mortem, and the tenth is still normal as determined by repeated rhinoscopic examinations.

Experiment 4

Fifteen rats and nine 21-day-old pigs were used in this experiment. Seven rats received nothing and were used as uninfected controls. Eight rats were instilled with pooled washings and scrapings from the noses of five pigs which had developed atrophy of the turbinates as a result of natural exposure in an infected herd.

Twenty-one days later all rats were killed, and the nasal scrapings from each group were pooled and suspended in physiological saline, centrifuged, and instilled into the noses of six pigs; four pigs receiving one instillation of nasal scrapings from the rats instilled with the atrophic rhinitis positive material and two pigs receiving the normal rat material. Three pigs were not instilled and were used as controls.

Results. One pig receiving the infective material died 15 days later. Both ventral turbinates were found to be residual on post-mortem examination. Histological examination of the affected tissues disclosed that the ventral turbinates contained no foundation of bone or cartilage, thus confirming the diagnosis of atrophic rhinitis. The three remaining pigs instilled with infective material and the controls had normal turbinates when examined post mortem on the sixteenth day of the experiment.

Experiment 5

Eight rats and six 15-day-old pigs were used in this experiment. Five rats received nothing and were used as uninfected controls. Three rats were instilled...
intranasally with fresh nasal scrapings from a pig which developed atrophy of the ventral turbinates following experimental infection.

On the 23rd and 27th days, respectively, after instillation, one rat from each group was sacrificed, and on the 30th day the remaining instilled rat and three control rats were killed, and the fresh nasal scrapings were suspended in physiological saline. These suspensions were centrifuged and instilled into the noses of four pigs; two pigs receiving the scrapings from the rats instilled with the atrophic rhinitis positive material and two pigs receiving the normal rat material. Two others received nothing and were used as controls.

Results. Twenty-five days after the first instillation of infective material one pig died from an unknown cause and the post-mortem examination disclosed typical lesions of atrophic rhinitis. Post-mortem examination of one uninstilled control pig which died the same day with a similar clinical appearance revealed normal turbinates. The three remaining control pigs and the one pig instilled with infective material were killed 89 days after the beginning of the experiment and were found to have normal turbinates.

Experiments 6 to 11

Six experiments were carried out which did not result in the transmission of atrophic rhinitis after passage of the infective agent(s) in the rat. Four of these involved fresh material similar to that used in the experiments just described, and two involved infective material stored in a dry-ice chest for 3½ and 11 months, respectively, before instillation into rats. In the first four experiments mentioned, the infective material was allowed to remain in the rats 28 to 248 days before it was instilled into the noses of pigs one to 16 days old. These pigs did not develop atrophy of the turbinates during a period of 50 to 110 days after instillation, as determined by rhinoscopic and post-mortem examinations.

Just before the pigs were to be instilled, the infected and control rats were killed by injecting ether into the heart or by ether inhalation. The head of each rat was skinned, flamed, and the nares opened dorsally by means of sterile scissors by an anterior-posterior cut in each nostril. The roof of the nasal cavity, including the septum, was removed and put in 2 to 3 ml. of sterile physiological saline in a 50-ml. round-bottomed centrifuge tube. The anterior of the nose of the instilled rats was examined for abnormalities which may have occurred following the introduction of the infective material. The side and floor of the nares were then scraped with sterile scalpels, and the scrapings transferred to the appropriate tube. After the pieces of tissue had been macerated and stirred in the saline for a few minutes by means of a wooden applicator or glass rod, the suspensions were centrifuged for three to five minutes at 3,000 r.p.m. About half of the supernatant was poured off, and the sediment examined for Trichomonas sp. by the methods previously described. About 0.25 ml. of the remaining sediment and saline were instilled into each nostril of the pigs which were to receive the rat material. Comparable uninstilled control pigs were put on experiment at the time these pigs received their first instillations.

All pigs were examined by means of the rhinoscope, and nasal washings were examined for protozoa by these same methods at intervals of about 30 days, be-
gaining 15 to 30 days after the experiments were started. The rhinoscopic observations were checked by post-mortem examination when a pig died or was sacrificed at the close of an experiment. An attempt was made to isolate a bacterial agent from the shrunken turbinates which developed in the pig instilled with infective material in experiment 3, using standard culture and isolation techniques. In a few cases pieces of nasal mucosa and the underlying bony structure were preserved in 10 percent formalin and were subjected to histological examination.

The infective material used in the last two experiments had produced characteristic lesions of atrophic rhinitis after direct instillation into the noses of pigs. However, when instilled into the nose of 25- and 27-day-old pigs after 21 to 42 days in rats, this material failed to produce lesions of atrophic rhinitis during a period of 52 and 85 days, respectively, as determined by rhinoscopic and post-mortem examinations.

**DISCUSSION**

The data in Table I show that two-thirds of the pigs instilled with material from the noses of white rats containing the causative agent(s) of atrophic rhinitis developed characteristic lesions of the disease. These pigs were 26 days old or younger, and the lesions were first observed rhinoscopically 15 to 45 days after the first instillation of infective material. This interval was not correlated with the number of instillations which varied from one to nine. The number of positive cases recorded would probably have been greater if experiments four and five had not been terminated so soon after the discovery of atrophy of the turbinates in the one instilled pig in which it occurred. The negative results obtained with material remaining 25 days or longer in the nose of the rat indicated that the maximum period during which the agent(s) involved in the present experiments remained infectious in rats was probably nearer 21 than 35 days.

These findings are significant in the light of Schofield's recent report (6) of an outbreak of atrophic rhinitis, in which the domestic cat was found to be a carrier of the causative agent(s). This investigator reported that the instillation of gross material from the respiratory tract of these cats into the noses of pigs was followed by the development of atrophic rhinitis. The present findings support Schofield's observations that atrophic rhinitis can be transmitted by a non-porcine carrier, and they also establish the possibility that there are natural carriers of the disease other than cats.

In view of Schofield's work, the reports of Thunberg and Carlstrom (7), Jones (8), and the present writers, of the close association of cats showing symptoms of a purulent rhinitis and pigs affected with "sneezing disease" or "atrophic rhinitis" assume greater significance since these animals may conceivably play an important role in the transmission of the disease.

The results obtained after passage of the infective material in the rat compare favorably with hitherto unpublished data obtained by two of us (JSA and FLE) in five experiments using similar infective material instilled directly into the noses of pigs three to 16 days old. Eight of twelve instilled pigs involved in these experiments also developed characteristic lesions of atrophic rhinitis. These lesions were first observed by means of the rhinoscope 24 to 51 days after the first in-
stillation of infective material. As in the experiments with rats, this interval was not correlated with the number of instillations administered, which also varied from one to nine.

The shorter interval (15 to 45 days) required for the lesion to develop following the instillation of the infective material after passage in the rat may indicate that the infectivity of the agent(s) may have been increased by this procedure as compared with material instilled directly into pigs.

The failure of quick-frozen material, which was infective for pigs by direct transfer, to produce atrophic rhinitis after passage in rats was not satisfactorily explained.

The Canadian workers, Gwatkin, Dzenis and Byrne (9) reported the production of atrophy of the turbinates in swine by the instillation of pure cultures of a strain of Pasteurella multocida obtained from pigs affected with atrophic rhinitis. Later Gwatkin and Dzenis (10) reported the isolation of P. multocida from six field cases and the production of atrophic rhinitis in baby pigs with pure cultures of this organism.

Heddleston, Shuman and Earl (11), however, isolated P. multocida from only about eight percent of the affected pigs in the Beltsville herd. The present authors also failed to produce lesions of the disease with a strain of P. multocida preserved by the lyophile process, which had been isolated from the nose of a pig with advanced atrophy of the turbinates.

Gwatkin, Dzenis, Greig, and Grinevitsch (12) obtained a moderate degree of atrophy in two of three seven-day old pigs instilled with nasal scrapings of the eighth rabbit passage of material which had originally been very active in pigs. We were unable to use rabbits in transmission studies with atrophic rhinitis positive material from the Beltsville herd because the five rabbits used in three trials died within three to nine days after intranasal instillation. Although the cause of death was not determined, it was assumed to be due to the infectious material instilled.

Schofield (loc. cit.) reported isolating a strain of P. multocida from the respiratory tract of cats affected with rhinitis which produced atrophy of the turbinates when instilled into the noses of pigs. In our experiments, this organism was recovered from only two of the many groups of rats instilled with infective material. Attempts to produce atrophy of the turbinates in pigs with material from the nose of the rat after instillation with these organisms were unsuccessful. The apparent absence of P. multocida from the pigs of the Beltsville herd which were affected with rhinitis, the inability of rabbits to survive infection with our material, and our inability to produce the disease with P. multocida, indicate that two different agents may be causing similar lesions in the two localities and support Switzer's contention (13) that several unrelated agents may cause atrophy of the turbinates in swine.

No pathological changes were observed in the noses of the rats instilled with infective material from the noses of pigs affected with atrophic rhinitis. This finding is of interest in view of the observations of Gwatkin, Dzenis, and Byrne (loc. cit.) and Gwatkin and Dzenis (14), who reported the development of atrophy of the turbinates in rabbits about two weeks after instillation with P. multocida.
of porcine origin. The last-mentioned authors also produced typical atrophic rhinitis in four-week-old rabbits which were given one intranasal instillation of infective material from a pig experimentally infected with the disease. These workers also reported that the rabbits usually had more pus in their nasal passages than pigs, but decalcification of the turbinate structures was just as definite and the lesions were otherwise the same as those seen in naturally and artificially infected pigs. *P. multocida* was isolated from a large percentage of the affected rabbits.

Although *Trichomonas* sp. was recovered from the noses of the rats into which these protozoa were instilled and from the noses of some of the pigs, there was no indication of pathological changes in the rat nose or of atrophy of the turbinates in the pig which could be correlated with the presence of these organisms. This finding supports the work of Fitzgerald, Hammond, and Shupe (15), Levine, Marquardt, and Beamer (16), and Hansen and Flatla (17), who were unable to produce atrophy of the turbinates in pigs with pure cultures of these protozoa.

Of significance is the observation that nasal washings and scrapings capable of producing characteristic lesions of atrophic rhinitis in pigs did not necessarily originate in pigs having atrophy of the turbinates. This finding supports the statement of Shuman and Earl (18) that porcine carriers which do not develop atrophy of the turbinates, although in daily contact with affected members of the same herd, may be a factor in the transmission of the disease. On the other hand, the present authors (JSA-FLE) have demonstrated in one of their experiments on the direct transmission of atrophic rhinitis (*loc. cit.*) that the disease can be transmitted to pigs by infectious material taken from an experimentally infected pig before atrophy of the turbinates developed sufficiently to be visible by means of the rhinoscope.

The pigs developing shrunken turbinates as a result of experimental infection showed no external evidence of the changes taking place within the nose. The reason for the absence of the "crooked snout," which is a lesion commonly observed in field cases of atrophic rhinitis, has not been determined.

**SUMMARY**

Infective material causing atrophic rhinitis in the herd of swine at the Agricultural Research Center, Beltsville, Maryland, produced typical lesions of the disease after passage in the nose of the albino rat from nine days to approximately three weeks. The rat, therefore, may act as a carrier of the causative agent(s) of atrophic rhinitis from infected to uninfected premises.

**ACKNOWLEDGMENTS**

The authors wish to express their appreciation to Dr. W. T. Shalkop, Veterinary Pathologist, of the Animal Disease and Parasite Research Branch, ARS, Beltsville, Maryland, who described the histological changes which occurred in the nasal mucosa and turbinate bones of the experimental animals, and to Mr. Paul Dwaresky, formerly Bacteriologist, of the same organization, who did the bacteriological work.
LITERATURE CITED

REPORT OF COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

H. U. GARRETT, Des Moines, Iowa, Chairman; L. P. DOYLE, Lafayette Indiana; H. C. H. KERNKAMP, St. Paul, Minnesota; A. H. QUIN, Kansas City, Missouri; J. D. RAY, White Hall, Illinois; L. A. ROSNER, Jefferson City, Missouri

Your committee on Transmissible Diseases of Swine this year, as has been done in the past, polled the various states in order to obtain a more clear picture of swine diseases in all states. However, the Committee has not concerned itself with some of the diseases that are handled by other committees of the Association.

Of the forty-eight states sent questionnaires, forty-five responded, for which we say thank you.

GENERAL

The Committee was first concerned on the movement of hogs, both interstate and intrastate.

In the intrastate movement, we were concerned with the regulations required on hogs that moved through sale barns. We were also concerned with the state requirements on the feeding of garbage to swine.

The relationship of cooking garbage to other diseases besides vesicular exanthema was considered important.

We found that almost one and a half million swine have been moved interstate for other than slaughter this year in the twenty-eight states reporting.

Intrastate—twenty-nine states reported veterinary inspection at all sale barns. Fifteen reported that no inspection was maintained.

Twenty-two states require all hogs selling through sale barns for other than immediate slaughter to be vaccinated for hog cholera before leaving the barn. Twenty-one states do not have this requirement.

Forty-two states have a law requiring the cooking of garbage before it is fed to swine. Three do not have such a law.

Only one state of the forty-five reporting has had an outbreak of vesicular exanthema this year.

One question asked in the questionnaire was, “Has the cooking of garbage, in your opinion, lowered the incidence of disease in swine other than vesicular exanthema in your state?” Thirty-nine states indicated that it had, three replied that it had not; three gave no answer. An overwhelming majority favored cooking, advantageous aside from vesicular exanthema.

HOG CHOLERA

That age old disease of swine is still with us, and seems to be causing considerable trouble in many states.

Six states report hog cholera had increased. Fourteen reported no change, and twenty-two reported a decrease.

The question was asked percentage-wise of the use of serum and virus versus
vaccine for the prevention of hog cholera. The report varied from zero to 100 percent in different states for each product.

The average, as we have figured it percentage-wise from reports from sanitary officials, is 28 percent for serum and virus and 72 percent for vaccines.

Perhaps the switch of vaccination with live virus to the modified virus is an indication that the time is near for an active campaign for eradication of hog cholera.

Your Association has a committee on eradication of hog cholera which will no doubt give you more details.

**Erysipelas**

Twenty-four of the forty-five states reporting stated that erysipelas is a problem. Thirteen stated the disease is on the increase in their state, twenty-one declared the disease unchanged, and six indicated the disease had decreased.

Thirty-five states control the use of erysipelas culture, nine do not, two failed to answer this question.

Avirulent live culture is used in thirty-six states, erysipelas bacterin in ten.

To the question of results from either product, twelve states satisfactory, five were not, twenty-five failed to answer.

Your Committee recommends more research be channeled into many of the problems of erysipelas. With the newer immunizing agents now available, we believe that with a few more answers to the common problems of this disease that in the foreseeable future, the use of culture may be eliminated and that we can look forward to a better control of erysipelas and then to a more realistic goal, final eradication.

**Atrophic Rhinitis**

Your Committee deemed this disease important enough for special discussion elsewhere on this program, therefore we will not deal with other than the report taken from the questionnaire.

Fifteen states stated atrophic rhinitis is a serious problem in their states. Twenty-six states that it was not. Fifteen states indicated the disease is on the increase and twenty-three reported no change. Two states stated the disease to be on the decline.

Six states mentioned methods of control such as quarantine, sanitation and slaughter.

**Leptospirosis**

During the past few years the farm press has called its readers attention to leptospirosis, and justly so. It is not only another problem for the livestock industry but may become a significant human health hazard.

In the questionnaire sent to the various state officials, three stated that leptospirosis is a problem and twenty-seven that it was not. Four stated the disease is on the increase in their states. This no doubt is true in most states if we had the true picture. Blood samples sent to laboratories for other routine tests have
revealed many herds infected with leptospirosis that may not have been revealed for some time.

**TRANSMISSIBLE GASTROENTERITIS**

Transmissible gastroenteritis is another swine disease about which little is known. More research is needed, as no state sampled had what they called a satisfactory control program.

Thirteen states indicated the disease is a problem. Twenty-eight stated that it was not, and four did not answer that question.

The disease had increased in three states, decreased in four, unchanged in twenty-one, and fifteen states did not indicate.

**ENTERITIS COMPLEX**

The enteritis complex, which may be considered to cover a wide spread number of conditions in the intestinal tract of swine, is a problem in twenty-two states. The report shows eighteen considered it of minor importance and five failed to answer.

To the question of the incidence of the disease, six reported an increase, four a decrease, twenty-two unchanged. The others failed to answer.

Feeds containing antibiotics has no doubt favorably influenced the enteric disease problem, but more research will need to be made before a satisfactory control may be asured to the swine industry.

**RESPIRATORY COMPLICATIONS INCLUDING FLU**

Respiratory complications is another one of the problems which the swine industry has faced many years, without many answers and without the necessary research.

Eighteen states considered this disease complex a problem, while twenty did not. Twelve failed to answer. Twenty-three states declared no change in the incidence of the disease and eight saw an increase. Thirteen failed to answer.

Here again we must consider the influence, or the importance, antibiotics mixed with commercial feeds may have.

**EPERYTHROZOONOSIS**

Eperythrozoonosis was recognized in seventeen states reporting. It is not considered of importance in some states, while others believe it is becoming a major problem in swine husbandry. The disease is being recognized more and more in baby pigs as well as older animals. Subclinical cases prevail in most instances.

Recovered cases remain carriers of the parasite and are potential spreaders of the disease. Carrier sows are a source of infection for their pigs. Also, in the process of vaccination of a herd, the disease may be transferred on the hypodermic needle from a carrier animal to a susceptible one.

Too little is known about the epizootiology and economic importance of eperythrozoonosis in swine. Your committee feels that this disease is more widespread than is being recognized, and it poses a potential hazard in swine that
might be compared to that of anaplasmosis in cattle. Therefore, it deserves serious consideration.

CONCLUSION

It is germane to mention in any report on transmissible diseases of swine that our knowledge of viral pathogens affecting this species of animals is still far from complete. A classical example is virus pneumonia of pigs. Until the recent reports by Betts of England, this disease, shown to be present in an average fifty percent or more of swine at two of our leading slaughter centers, was not even recognized as a specific entity. While responsible for negligible death losses, the economic toll from virus pig pneumonia amounts to millions of dollars annually.

Within the past decade the new science of tissue culture, as well as numerous other advancements and technics aimed at isolating and identifying pathogenic and non-pathogenic viruses have been developed. Broader utilization of these new "tools" by federal and state animal disease research laboratories is urgently needed to clearly identify many as yet obscure outbreaks of diseases in swine, which by the laws of probability, are due to viruses as yet not recognized as specific entities.

Your Committee was interested in the condemnation of swine for disease at the time of slaughter and made an examination of the Summary of the Activities of the Meat Inspection Branch for the fiscal year 1956.

It was found that at all Federally inspected slaughtered houses 1621 swine were retained on antemortem inspection.

Upon postmortem 119,034 carcasses were condemned. Of this number, 14,214 were condemned for a transmissible disease.

Parts of carcasses condemned for various diseases and conditions on postmortem inspection totaled 2,175,698. Truly, a tremendous loss not only to the meat packer and the swine industry but to the consumer as well, and certainly, a high percentage of this loss could have been prevented if we had more research as to the cause and effect of transmissible disease of swine and thereby establish a better disease control program at the farm level.

With the establishment of a new and larger animal disease research laboratory in the midwest, we hope that many of our disease problems of today will be given careful consideration tomorrow, and with those answers a better control program at the farm level.

RECOMMENDATION

1. Your Committee recommends that the United States Livestock Sanitary Association continue to appoint a committee on transmissible diseases of swine. We also wish to compliment the committee on eradication of hog cholera. We believe the time is near when an all out educational campaign can be instituted for the eradication of this disease.

2. We recommend that more research be conducted on transmissible diseases of swine.

3. We recommend that a very extensive research be made on virus diseases of swine. Especially as to possible reservoirs of carry over and transmission and a method of breaking the cycle.
REPORT OF THE SWINE BRUCELLOSIS COMMITTEE

R. W. CARTER, Columbia, South Carolina, Chairman; R. FENSTERMACHER, St. Paul, Minnesota; J. W. GREEN, Indianapolis, Indiana; L. M. HUTCHINGS, Lafayette, Indiana; C. A. MANTHEI, Beltsville, Maryland; W. R. TEETER, Dover, Delaware

Little progress can be reported in the control and eradication of swine brucellosis. Most states have adopted programs based on the recommendations of this committee, but none are adequately supported. Limited use of these programs indicates they have value in controlling infection in the herd but could be materially improved by the assistance of a more specific diagnostic test for detecting infection in individual animals. Furthermore, no state official charged with the responsibility of disease control has sufficient funds to conduct an extensive swine brucellosis control program.

A few years ago, Eisle and McCullough reported that Brucella abortus and Brucella melitensis were isolated from an appreciable percentage of swine samples in Chicago packing houses. Although most husbandry practices are such that it may not be necessary to eradicate swine brucellosis in order to control bovine brucellosis, the potential of swine as a reservoir of all three species of brucella cannot be overlooked in an over-all brucellosis eradication program. The entire livestock industry, therefore, must be concerned in a swine brucellosis control program and undoubtedly will give support and assistance to this endeavor. The swine industry, however, must take an active and aggressive leadership in solving their problem, and only after it has displayed this interest will the necessary programs, finances, and research be forthcoming.

Past history has shown that before an over-all disease eradication program can be successfully inaugurated, it has been necessary to establish some small area trials. With this in mind, your Committee on swine brucellosis recommends the establishment of four selected areas for the control and eradication of swine brucellosis. Such areas should be not less than a township nor more than a county in size. All known sound procedures for swine brucellosis control and eradication should be applied on this limited area basis.
ENZOOTIC VESICULAR STOMATITIS

R. P. HANSON AND L. KARSTAD

Vesicular stomatitis is now known to have reappeared in the United States since the time of the Revolutionary War. In some parts of southern United States the disease is seen each year and in parts of northern and western United States it is seen less frequently, sometimes 10 or more years apart (4). The behavior of the disease in these two regions is quite different. Only in the south does it appear as an enzootic; only in the south does the virus regularly attack swine. Our study of the epizootiology of vesicular stomatitis has been confined to two areas: one, in northwestern Wisconsin, typical of the region in which the infrequent epizootics occur, and one in southeastern Georgia, typical of the region in which the disease reappears annually.

In northwestern Wisconsin disease outbreaks occurred in 1926, 1937 and 1949, involving many hundreds of cattle and horses each time (1). The first cases were observed in July or August of the epizootic years, and the last in early October. While studies were not conducted in the years following the 1926 and 1937 outbreaks, herds were kept under observation in the years succeeding 1949 and new cases did not occur. Antibodies have persisted in recovered cattle but all replacement animals have been negative.

Because vesicular stomatitis, as it exists on the farms in enzootic areas, may be insidious in nature, it is very difficult to estimate the number of cases that occur each year. The number of premises on which positive diagnoses were made during the years 1952 to 1954 were reported by Schoening (7) in a paper presented to the United States Livestock Sanitary Association at Omaha in November 1954. Including those years and the 1955 season, vesicular stomatitis in swine has been diagnosed in North Carolina in 1953, 1954 and 1955; in Georgia in 1952, 1953 and 1955; in South Carolina in 1955; in Florida in 1954; and in Louisiana in 1954 and 1955. The enzootic may cover most of the lower coastal plain in southeastern United States. Only in the vicinity of the Altamaha River drainage in Georgia has the enzootic been studied in sufficient detail to determine that approximately 50 percent of the animals carry virus neutralizing antibodies.

The Atlantic Coastal Plain extends from Virginia southward to Florida—a strip of land about 100 miles wide and 800 miles long. The flat plain is a patch-work of pine woods and cypress swamps, crossed every few miles by sluggish streams. The soil grades from dry sand in the higher portions to black loam and saturated muck soils in the swamp lands. The Coastal Plain can be divided into an upper and lower portion. The latter has an altitude of less than a hundred feet and lies within 50 miles of the ocean. The climate of this lower Coastal Plain differs from that of the upper plain and the still higher piedmont. The growing season at Glennville on the lower coastal plains is about 45 days longer than at Athens on the piedmont, and the average January temperature is 10 degrees higher; the average July temperature is about five degrees higher. The precipitation, however, is less at Glennville—48 inches of rainfall as compared to 56 on the piedmont. The flora
ENZOOTIC VESICULAR STOMATITIS

is characterized by palmettoes, live oak trees adorned with Spanish moss, cypress and blue gum trees, all of which are rare or absent on the upper coastal plain. The fauna has some unique species. Among the 45 mammals reported by Hamilton (3) to live in southern Georgia, 31 percent are a different subspecies than the representative on the piedmont, and 27 percent do not have a species representative on the piedmont. In other words, about 50 percent of the mammals are different. This is also true of the lower animals; the frogs, the snakes and the arthropods.

Animals on the eleven farms in the vicinity of Glennville, Georgia were bled and tested for vesicular stomatitis antibodies. The results can be summarized briefly: From 23 to 80 percent of the animals on each farm had antibodies. All of the 10 horses, 57 percent of the 59 pigs and 50 percent of the 116 cows on the eleven farms had antibodies. Goats and dogs were negative in all cases. Man and other animals were not tested. The age of the animals is significant in evaluating the antibody titer. Seventy percent of the cattle of milking age (three to 10 years old), 35 percent of the heifers (four to 24 months old) and 70 percent of the young calves less than three months of age possessed antibodies which neutralized vesicular stomatitis virus. The antibody in calves is undoubtedly a reflection of the colostral transfer. Experimental studies on vesicular stomatitis in Wisconsin and research by workers in Africa on rinderpest indicates that antibodies to certain virus infections may be retained by the calf and can be detected in significant titer for three to four months after birth. Presence of antibodies in animals from all age groups is further evidence of annual recurrence of vesicular stomatitis in southern Georgia. This is very different from the situation in northwestern Wisconsin where the disease has occurred at about 10 to 12 year intervals, and antibodies are found in one age group only.

Vesicular stomatitis appears in the enzootic area each May or June after a short period of rain and warm weather. The first cases of each outbreak season are seen in hogs which have had access to swampy land along creeks or rivers. Later cattle may be found affected and, as the season continues, animals farther away from the swamps may also be involved.

Farms on which vesicular stomatitis appears in hogs in May or June differ ecologically from farms on which the disease may appear later in the season or not at all. In every instance investigated the pigs affected early had access to wet lands, either a small stream or a swampy pool, sheltered by a cover of trees. The fauna is richer in such an environment than in the open dry, sandy pine lands which are usually free of the disease. It is often noticed that on adjoining farms the one with the wet-land hog pasture had infected or recovered pigs and the other with the dry-land pasture had swine free of infection.

Although the disease has appeared each summer, there remained a period in the winter of six to seven months in which the disease was not seen in any part of the enzootic area, the period of October to May. Our knowledge of the physical stability of the virus indicates that it would be impossible for the virus to persist outside of the living cell for more than a few days. A reservoir capable of harboring the virus is the only simple explanation.

The possibility of a reservoir of the virus in a wild animal population has been under investigation. Among many species of mammals trapped, bled and tested
for the presence of antibodies, only four have been found to possess significant
titers of vesicular stomatitis antibody. Forty-eight percent of some 59 raccoon
possessed high neutralizing antibodies, 60 percent of 25 deer, 83 percent of 34 feral
swine and 37 percent of eight bobcats (5). The infection was studied in five deer
and 25 raccoon. The deer were susceptible to vesicular stomatitis introduced
through abrasions on the tongue and muzzle. Vesicles developed; there was a
thermal response and a period of transient depression. The virus was reisolated
from the mouth lesions. The animals recovered rapidly and developed high neu-
tralizing antibodies. The raccoon exposed by placing virus on the abraded snout
and tongue surfaces or by feeding infective material developed an inapparent in-
fection with subsequent high-level antibody response.

On the basis of our observations, we must conclude that deer are highly suscep-
tible to infection with the virus of vesicular stomatitis, that such infections are of
moderate severity and that recovery is usually rapid and complete. The lesions
observed were of a milder nature than those usually seen in cattle, yet it took less
virus, one-thousandth as much, to produce a lesion in a deer with a Georgian strain
of vesicular stomatitis virus, New Jersey type, than to produce one in cattle. Poss-
ibly other strains of virus would titrate higher in cattle than in deer. Because the
clinical manifestations of vesicular stomatitis infection in deer are of an acute,
short-term nature and because of the rapid development of high-level virus neu-
tralizing antibody in the blood, it is unlikely that deer are important as long-term
carriers or reservoirs of the virus; however, they may play an important part
adding fuel of susceptible animals to the fire of the summer epizootic.

There have been numerous reports in the enzootic area of deer with sore mouths
or hoofs, Dr. F. C. Randall and the state wildlife ranger, W. Q. Smith, reported
seeing such deer killed by hunters. Some of the carcasses were in such poor con-
dition that they were discarded. Several cases of deformed hoofs were seen in No-
vember of 1955 and, at about the same time, Captain Chapman, post veterinarian
at Fort Stewart, Georgia, was notified that a farmer’s dog had caught and killed
two deer which were in poor condition and showed foot and mouth lesions. He made
a special effort to investigate this report but the farmer had already burned the
carcasses.

Vesicular stomatitis infection of the raccoon although usually clinically inap-
parent and of short duration was also followed by rapid production of virus neu-
tralizing antibodies. All attempts to recover virus following exposure, with one
exception, at 60 hours, gave negative results. It is unlikely that the raccoon is
important as a long-term carrier or reservoir of the virus, however, the raccoon
may have a role in the perpetuation of the disease in an enzootic area.

Recognition of several susceptible wild animal species makes it easier for us to
understand how vesicular stomatitis can persist in an area in the presence or ab-
sence of domestic livestock. One can visualize the natural persistence of sylvan
vesicular stomatitis in wild mammals and as yet undetermined reservoir hosts, a
cycle in which domestic animals enter on a mere chance basis. There would seem
to be little possibility of eradicating such a disease.

The means of natural transmission of vesicular stomatitis has not been estab-
lished. Attempts to transmit vesicular stomatitis to cattle by feeding virus have
been unsuccessful in almost all instances. Work in our laboratory by Ferris (2)
and by Roberts (6) has shown that transmission to cattle could be by insects acting as mechanical carriers. Research has been directed to determining the role of diptera in the summer epizootic. Other methods of transmission are not to be ignored. It is evident from experimental infections that raccoon and swine may become infected by ingestion of vesicular stomatitis virus. In these cases, it has not been determined whether small oral abrasions or coincidental invasions of the host tissue by metazoan parasites are essential for infection to occur.

In some preliminary experiments in which vesicular stomatitis infected chicken embryos were fed to hogs, inapparent infections were induced and the exposed hogs developed serum neutralizing titers within two weeks after ingestion of the virus. When embryonated ascarids were fed along with the virus, vesicles developed on the snout and respiratory signs were seen presumably due to the migration of virus contaminated ascarid larvae to the lung. Virus fed to raccoons in the form of infected hens' eggs produced antibody response without the development of apparent lesions or other signs of disease. It is probable that the oral route is the natural avenue of transmission in the raccoon.

The recognition that raccoon and feral hogs are regularly exposed to vesicular stomatitis in the enzootic area that they may be infected by oral route and that swine at least are one of the first to show signs of disease in the spring suggest a search for the reservoir hosts among animals eaten by swine and raccoon. Frequent exposure by this route and development of immune animals would also explain the relative infrequency of the occurrence of vesicular lesions in swine. In the outbreak which occurred among approximately 800 swine on the premises of Mr. Adamson, only three clinically affected animals were seen. These individuals were in different age groups on widely separated parts of his estate. All of these animals, however, had access to swampy wooded pastures and all three which were clinically affected developed lesions within a two week period of time. There was a history of a few similar cases being seen about the same time of year for the past several years. It is quite possible that infection of cattle, deer and horses occurs by means of some vector after the virus has been reawakened in orally infected raccoon and swine at the beginning of the outbreak season.

Serious consideration has been given to the possibility of the predator-prey relationship in the transmission of vesicular stomatitis, particularly as a means of producing the first case of each season. The diet of the feral hog or the domestic hog on free range has not been described for the United States. Stegeman (8), however, reported that the wild boar in Tennessee ate worms, insects, crayfish, frogs, salamanders, lizards, snakes, small mammals, a chipmunk, young birds and bird eggs. Apparently everything is eaten either alive or as carrion. A man who observed hundreds of feral pigs in southern Georgia reported that they fed heavily on earthworms, also they took frogs, snakes, turtles and crayfish. A pig shot in July, 1955 had 200 earthworms in its stomach. Like the feral pig, the raccoon is omnivorous and its diet varies with the season and the locality.

Attempts were made beginning in the summer of 1955 to collect materials suspected of harboring virus. Invertebrate and vertebrate animals were obtained from premises where vesicular stomatitis outbreaks had occurred or were in progress. Preference was given to those species that had a known contact relationship with one or more of the recognized hosts and to those species which were most abundant
in the outbreak areas during the time when the outbreak occurred. Vesicular stomatitis virus was not isolated from materials collected in the outbreak area during the summer of 1955. Several virus-like agents, however, were recovered from these animals. One, in particular, was from the earthworm *Pheretima diffringens* which is an important item in the diet of the pig.

Another study which has influenced our thinking on the reservoir host is the susceptibility of frogs to vesicular stomatitis virus. In an initial experiment, 12 frogs were exposed to an embryo passage of a strain of virus isolated in Georgia, six received a half ml of infected allantoic fluid by mouth and six were inoculated subcutaneously with a tenth ml of the same material. Frogs were held in moist atmosphere at $4^\circ$C in a condition of hibernation. One frog of each group was sacrificed each week for six weeks. Lungs, liver, spleen, kidney and bladder, ova and the skin were harvested for the presence of virus by intraallantoic inoculation. No lesions or signs of disease were observed in the frog but virus was recovered from the lungs of orally exposed frogs at one, two and three weeks after inoculation and from the lungs of subcutaneously inoculated animals at two, three and four weeks postinoculation. The group inoculated subcutaneously also yielded virus from the skin one week after inoculation. Subsequent experiments have largely confirmed this observation and extended the period during which the virus is carried by the frog.

At the present we may speculate that the enzootic vesicular stomatitis cycle in southeastern Georgia may be something like this: The virus carried over the winter season in a cold-blooded animal is ingested in the spring by a raccoon or pig who develops the infection. A vector picks up the virus from the diseased pig or raccoon and carries it to deer, cattle and horses. Vesicular stomatitis is maintained during the summer season in these species, then each fall it is carried over again by the cold-blooded animal reservoir. It must be emphasized that this is speculation. Speculation is stimulating to an investigator provided he continues his search for the real story.

REFERENCES

A TYPING STUDY OF VESICULAR STOMATITIS VIRUS FIELD SAMPLES OF SWINE ORIGIN

A. A. Holbrook, D.V.M., J. N. Geleta, D.M.V., M.S., and
W. C. Patterson, V.M.D.*

The possibility of virus variation has an important bearing on epizootiology. This is exemplified by the variants which have been found in foot-and-mouth disease (1) and in vesicular exanthema (2). It is difficult to separate a true genetic mutation from pseudo-variations related to other changes such as increased or decreased infectivity.

Schoening and Crawford (3) first definitely diagnosed vesicular stomatitis in North American swine in 1943. This outbreak was confined to a veterinary biological establishment in Kansas City, Missouri. Shahan, Frank, and Mott (4) designated this virus as Missouri strain of New Jersey virus and found it to be the same immunological type with the same pathogenic qualities as other New Jersey viruses from cattle and horses.

In the typing of vesicular diseases at the Beltsville laboratory, a large number of virus samples of swine origin have been received since 1952 from field outbreaks. These samples have been typed as vesicular stomatitis of the New Jersey type. Vesicular stomatitis is produced by only two serologically distinct virus types: New Jersey and Indiana. None of the samples of swine origin have proved to be of Indiana type. This collection of viruses presented an opportunity for a comprehensive study of relationships between viruses of swine origin from the field and known viruses previously used in other laboratory work. Eight viruses of swine origin from five States, covering a collection period of four years, and the four viruses which had been used most commonly in other laboratory studies of vesicular stomatitis were chosen for comparison.

MATERIALS AND METHODS

Procedures used to maintain quarantine have been described in a previous paper (5). Twelve virus samples, which had previously been determined to be New Jersey-type vesicular stomatitis, and which had been stored in a dry-ice chest at the Beltsville laboratory, were used in this study. The samples are as follows:

1. Hazlehurst: FS-30; May 1, 1952, Hazlehurst, Georgia. Epithelial tissue from swine involved in the original outbreak, in which both swine and cattle were affected.

2. Ponce de Leon: FS-257; July 1954, Ponce de Leon, Florida. Epithelial tissue from swine involved in the original outbreak, in which only swine were affected. In the differential tests in animals at the time of the outbreak, swine and the horse developed vesicles but the cow remained negative.

3. Bordelonville: FS-33; May 1955, Bordelonville, Louisiana. Epithelial tissue from swine in the original outbreak, in which only swine were affected.

* From the Animal Disease and Parasite Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
HOLBROOK, GELETA, AND PATTERSON

(4) Holly Hill: FS-342; June 1955, Holly Hill, South Carolina. Epithelial tissue from swine involved in the original outbreak, in which only swine were affected.

(5) Jesup: FS-409; September 1955, Jesup, Georgia. Epithelial tissue from a hog that died while being taken to a veterinary hospital. Vesicles were observed on feet and snout of the dead hog. The affected feet and snout were cut off and placed in a refrigerator. Later the vesicle coverings were harvested and sent to the Beltsville laboratory for diagnosis. This animal was the only one affected on the premise.

(6) Glennville: FS-365; July 1955, Glennville, Georgia. Epithelial tissue from swine involved in the original outbreak, in which only swine were affected.

(7) Brantley: FS-140; May 1953, Brantley, Georgia. This outbreak involved swine only. A test pig was scarified on the snout and placed in contact with the affected swine. Vesicles developed on the seventh day. The vesicle coverings from this test pig were used in this work.

(8) Roper: FS-350; June 1955, Roper, North Carolina. This outbreak involved swine only. Test animals were inoculated for a differential diagnosis. The cattle and horse remained negative, but the pigs developed vesicles at 135 hours. The epithelial tissue harvested from these test pigs was used in this work.

(9) Concan: FS-2; June 1949, Concan, Texas. Material harvested from the dental pad of a cow involved in the original infection was kept in a dry-ice chest until passed to a cow in June 1951. A good yield was obtained, and part stored in a dry-ice chest until used in this work. Passages from the other portion have been used in a great deal of typing and experimental work on vesicular stomatitis.

(10) Missouri: This virus was obtained from a spontaneous outbreak in swine in a veterinary biological establishment in Kansas City, Missouri. Guinea pig pads were received from Drs. Schoening and Crawford in August 1943. Subsequently 66 animal passages were made and the harvest from guinea pigs inoculated March 13, 1954, was stored in the dry-ice chest which was used in this study.

(11) Teasdale: FS-15; November 1949, Teasdale, Utah. This outbreak affected both horses and cattle. The epithelial tissue received was from the upper lip and dental pad of a cow. It was passed three times in cattle, the last on December 7, 1950.

(12) Saratoga: FS-14; October 1949, Saratoga, Wyoming. This outbreak affected both horses and cattle. Test animals for a differential diagnosis were inoculated with material harvested from the tongue of a yearling steer. The material used was the epithelial tissue sent in by Doctor Leiby in November 1949 from the test horse used in diagnosis.

The virus materials of Concan, Missouri, and Teasdale origin were unground vesicle coverings in sealed vials. The remainder had been held in the original 50 percent buffered glycerine phosphate as received from the field.

For preparation of inocula, 10 to 20 percent (by weight) suspensions were made by grinding the epithelial tissue in cooled mortar, using as an abrasive sterile ground glass. The epithelial paste was diluted with phosphate buffered saline (pH 7.6), and 2 mg./ml. dihydrostreptomycin added. This suspension was centrifuged at 2,000 r.p.m. for 10 minutes, and the supernatant liquid used.

Swine were Landrace crossbreeds secured from the Animal Husbandry Research Branch, ARS, Beltsville, Maryland, averaging about 175 pounds. The temperature
of all swine was taken prior to inoculation to establish the normal temperature patterns. During the experiment the temperatures were taken twice a day, and the swine were observed for development of lesions. All challenges to ascertain immunity in the swine were done by scarifying the snouts and interdigital spaces of the two front feet and rubbing a thick paste of vesicle coverings, previously harvested from New Jersey-Concan-infected animals, into the scarified areas. Normal animals were used each time to check the virulence of the inoculum. In all experiments strict quarantine and disinfection procedures were maintained between pens.

Passage Swine

Each of the 12 virus samples was made into a 20 percent suspension and inoculated intradermally into the snout and the interdigital spaces and coronary bands of all four feet of two pigs. Vesicle coverings were harvested and stored in dry-ice chest for further use.

Test Swine

Vesicular material from passage swine of each virus was inoculated intradermally into the snouts of four pigs. These pigs were observed for temperature rises, lesions, generalization, and other pathogenic changes. Serial bleedings were taken, and sera stored for further study by serum neutralization. Three of the pigs in each group were challenged with New Jersey-Concan virus three weeks post-inoculation. One pig of each group was held for further serial bleedings.

Chickens

Three adult New Hampshire Red chickens were inoculated by tunneling the epithelium on the dorsum of the tongues at the same time the test pigs were inoculated. Three weeks post-inoculation all chickens were challenged with known New Jersey-Concan virus. Serial bleedings were taken for serum-neutralization studies.

Guinea Pigs

To produce Indiana- and New Jersey-type immune guinea pigs, five weeks prior to use groups of 75 guinea pigs were inoculated by intradermal tunneling of the plantar pads. Only those guinea pigs on which vesicles covered the entire pads in 48 hours were saved for future use. Each of the 12 viruses were inoculated by intradermal tunneling into the plantar pads of 10 normal guinea pigs, five Indiana-type immune guinea pigs, and five New Jersey-type immune guinea pigs. All the normal guinea pigs were positive, and the vesicle covers were harvested from each of the 12 groups and stored separately in the dry-ice chest. Four weeks post-inoculation these separate vesicular harvests were used to hyperimmunize five guinea pigs of their corresponding groups by injecting 0.25 ml. of a 10 percent suspension into each plantar pad. Seven days later the guinea pigs were bled, and hyperimmune serum was prepared for each group. The other five guinea pigs in each group were challenged by scarification with a New Jersey-Concan virus paste four weeks
post-inoculation. All guinea pigs were periodically observed for both primary and secondary lesions.

**Embryonating Chicken Eggs**

Fertile chicken eggs which had been incubated for eight days were inoculated in the allantoic cavity with 0.2 ml. of a 10 percent suspension of the material harvested from the passage pigs. Four passages were made using chorio-allantoic membranes diluted with amnio-allantoic fluids. The eggs were candled twice a day, and all deaths between 12 and 96 hours recorded. On the fourth passage the membranes and fluids were harvested separately. The membranes were ground, diluted 1:15 with the fluids, centrifuged for 10 minutes at 3,000 r.p.m., and stored in the dry-ice-chest until needed for antigens in the complement-fixation test.

**Serum Neutralization**

Serum neutralization was conducted for the detection of immune antibodies in the sera of all test swine and all groups of chickens. The samples used were collected three weeks post-inoculation. The method described by Cunningham (6) was adapted to use with vesicular stomatitis virus: eight-day-old chicken embryos were inoculated with 0.15 ml. of a mixture of equal parts of tenfold dilutions of 20 percent egg membrane New Jersey-Concan virus suspension and 1:5 dilution of each of the sera. Deaths within 72 hours were counted as significant.

**Complement-Fixation**

Complement fixation for detection of variation as described by Traub and Mohlmann (7, 8) for foot-and-mouth disease was adapted to use with vesicular stomatitis. The hyperimmune guinea pig serum of each of the 12 virus groups was tested for effectiveness in dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320 with egg membrane antigens previously prepared for each of the 12 viruses. Comparison was based on the last dilution, in which 100 percent fixation occurred.

**RESULTS**

Approximately the same amount of material was harvested from the passage pigs of each group. In the test swine (Table I), primary vesicles developed on all pigs. The time of vesiculation varied from 24 to 96 hours following inoculation, with the Hazlehurst and Holly Hill groups having the shortest incubation period and the Concan and Saratoga groups having the longest. Secondary vesicles on the feet developed in all groups except Brantley, Concan, and Saratoga. All swine challenged at three weeks’ convalescence with an active laboratory Concan virus were negative. The serum neutralization titers LD₅₀ of 10 of the groups were within one log dose of that found in the homologous Concan serum. Missouri serum was 1.6 log doses less, but was still considered diagnostic.

All chickens (Table II), developed vesicles on the dorsal surface of the tongue but did not show any evidence of a systemic reaction. When challenged with an active Concan virus, all chickens except two in the Saratoga group remained negative. On the neutralization test with chicken serum, the sera of the Jesup, Glennville, Roper, and Saratoga groups had a titer too small to be diagnostic but showed
TABLE I
Test Swine Inoculated with Material Harvested from Passage Swine

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primary Vesicles on Snouts</th>
<th>Secondary Vesicles on Feet</th>
<th>Challenge with Concan Virus</th>
<th>LD₅₀ Serum Neutralization Titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazlehurst</td>
<td>24†</td>
<td>3/4‡</td>
<td>0/2‡</td>
<td>4.4</td>
</tr>
<tr>
<td>Ponce de Leon</td>
<td>24-48</td>
<td>3/4</td>
<td>0/2</td>
<td>3.6</td>
</tr>
<tr>
<td>Bordelonville</td>
<td>48</td>
<td>2/4</td>
<td>0/2</td>
<td>4.2</td>
</tr>
<tr>
<td>Holly Hill</td>
<td>24</td>
<td>3/4</td>
<td>0/2</td>
<td>4.2</td>
</tr>
<tr>
<td>Jesup</td>
<td>24-48</td>
<td>3/4</td>
<td>0/3</td>
<td>4.9</td>
</tr>
<tr>
<td>Glennville</td>
<td>48</td>
<td>4/4</td>
<td>0/3</td>
<td>4.8</td>
</tr>
<tr>
<td>Brantley</td>
<td>48</td>
<td>0/4</td>
<td>0/3</td>
<td>4.6</td>
</tr>
<tr>
<td>Roper</td>
<td>48</td>
<td>2/4</td>
<td>0/3</td>
<td>4.5</td>
</tr>
<tr>
<td>Concan</td>
<td>48-72</td>
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<td>0/4</td>
<td>4.6</td>
</tr>
<tr>
<td>Missouri</td>
<td>24-48</td>
<td>4/4</td>
<td>0/4</td>
<td>3.0</td>
</tr>
<tr>
<td>Teasdale</td>
<td>24-48</td>
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</tr>
<tr>
<td>Saratoga</td>
<td>48-96</td>
<td>0/4</td>
<td>0/4</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Three weeks postinoculation.
† Hours following inoculation.
‡ Numerator indicates number of swine positive; denominator indicates number of swine in group.

TABLE II
Adult Chickens Inoculated with Material Harvested from Passage Swine

<table>
<thead>
<tr>
<th>Virus</th>
<th>Vesicles on Dorsum of Tongue</th>
<th>Challenge with Concan Virus</th>
<th>LD₅₀ Serum Neutralization Titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazlehurst</td>
<td>24†</td>
<td>0/3‡</td>
<td>3.2</td>
</tr>
<tr>
<td>Ponce de Leon</td>
<td>48</td>
<td>0/3</td>
<td>2.2</td>
</tr>
<tr>
<td>Bordelonville</td>
<td>48</td>
<td>0/3</td>
<td>3.6</td>
</tr>
<tr>
<td>Holly Hill</td>
<td>24</td>
<td>0/3</td>
<td>3.2</td>
</tr>
<tr>
<td>Jesup</td>
<td>24</td>
<td>0/3</td>
<td>1.3</td>
</tr>
<tr>
<td>Glennville</td>
<td>24</td>
<td>0/3</td>
<td>0.8</td>
</tr>
<tr>
<td>Brantley</td>
<td>24</td>
<td>0/3</td>
<td>2.0</td>
</tr>
<tr>
<td>Roper</td>
<td>24</td>
<td>0/3</td>
<td>1.1</td>
</tr>
<tr>
<td>Concan</td>
<td>48</td>
<td>0/3</td>
<td>3.6</td>
</tr>
<tr>
<td>Missouri</td>
<td>48</td>
<td>0/3</td>
<td>2.3</td>
</tr>
<tr>
<td>Teasdale</td>
<td>48</td>
<td>0/3</td>
<td>3.8</td>
</tr>
<tr>
<td>Saratoga</td>
<td>48</td>
<td>2/3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Three weeks postinoculation.
† Hours following inoculation.
‡ Numerator indicates number of chickens positive; denominator indicates number of chickens in group.

some protection. The other eight groups, although lower than the corresponding swine titers, were nevertheless high enough to be considered diagnostic.

In the guinea pigs (Table III), similarity was shown with all viruses by producing vesicles in 44 hours on all normal guinea pigs, the failure of any to produce
TABLE III
Guinea Pigs Inoculated with Material Harvested from Passage Swine

<table>
<thead>
<tr>
<th>Virus</th>
<th>Normal Guinea Pigs</th>
<th>New Jersey Immune Guinea Pigs</th>
<th>Indiana Immune Guinea Pigs</th>
<th>Convalescent Guinea Pigs Challenged with Concan Virus*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazlehurst</td>
<td>10/10†</td>
<td>0/5†</td>
<td>3/5†</td>
<td>0/5†</td>
</tr>
<tr>
<td>Ponce de Leon</td>
<td>10/10</td>
<td>0/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Bordelonville</td>
<td>10/10</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Holly Hill</td>
<td>10/10</td>
<td>0/5</td>
<td>2/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Jesup</td>
<td>10/10</td>
<td>0/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Glennville</td>
<td>10/10</td>
<td>0/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Brantley</td>
<td>10/10</td>
<td>0/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Roper</td>
<td>10/10</td>
<td>0/5</td>
<td>4/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Missouri</td>
<td>10/10</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Teasdale</td>
<td>10/10</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Saratoga</td>
<td>10/10</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* Four weeks postinoculation.
† Numerator indicates number of guinea pigs positive; denominator indicates number of guinea pigs in group.

TABLE IV
Complement-Fixation Titration of the Twelve Sera with the Twelve Antigens

<table>
<thead>
<tr>
<th>Serum†</th>
<th>Serum Titer in Titration with Antigens*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazelhurst</td>
</tr>
<tr>
<td>Hazlehurst</td>
<td>160†</td>
</tr>
<tr>
<td>Ponce de Leon</td>
<td>80</td>
</tr>
<tr>
<td>Bordelonville</td>
<td>40</td>
</tr>
<tr>
<td>Holly Hill</td>
<td>80</td>
</tr>
<tr>
<td>Jesup</td>
<td>80</td>
</tr>
<tr>
<td>Glennville</td>
<td>80</td>
</tr>
<tr>
<td>Brantley</td>
<td>80</td>
</tr>
<tr>
<td>Roper</td>
<td>80</td>
</tr>
<tr>
<td>Missouri</td>
<td>40</td>
</tr>
<tr>
<td>Teasdale</td>
<td>160</td>
</tr>
<tr>
<td>Saratoga</td>
<td>40</td>
</tr>
</tbody>
</table>

* Egg-membrane antigen.
† Hyperimmune guinea pig serum.
‡ Last dilution in which 100% fixation occurred.
vesicles on the New Jersey immunes, and lack of vesicle production when challenge was made at three weeks with Concan virus on the convalescent guinea pigs of each group. Differences were observed in the production of vesicles on the Indiana-immune guinea pigs; some of the guinea pigs in all groups were positive, but only Bordelonville, Concan, Missouri, Teasdale, and Saratoga viruses produced lesions on all five guinea pigs.

In the complement-fixation test for strain specificity (Table IV), all the antigens were of relatively the same strength, but there was a difference of as much as two twofold dilutions in fixing ability of the sera. Hazlehurst and Missouri sera fixed all antigens in dilutions of 1:160, whereas Bordelonville and Concan sera fixed all antigens in dilutions of 1:40. When titrated with all the antigens, no serum was different by more than one twofold dilution. Seven of the sera had the same fixation with their homologous antigens as with the other 11 antigens. Roper and Glennville sera had less fixation with their homologous antigens than with some of the other antigens; whereas Brantley, Jesup, and Saratoga had more fixation with their homologous antigens than with some of the other antigens. On the type specificity tests, all 12 sera were negative to Indiana-type antigen in a 1:5 dilution.

**DISCUSSION**

The techniques employed in this work did not show sufficient antigenic variations to permit separation into strains. It is possible that further testing by serum neutralization and cross-protection in vivo could show differences great enough to designate strains.

It was observed that the four laboratory viruses produced lesions on all five of the Indiana-immune guinea pigs, whereas only the Bordelonville virus of the eight viruses of swine origin produced lesions on all five of the Indiana-type immune guinea pigs. This would indicate that the swine viruses as a group were not as pathogenic to the Indiana-type immune animals. It was also observed in the production of the egg membrane antigens that the four laboratory viruses adapted more readily, as indicated by a larger percentage of deaths in a shorter time; however, by the fourth passage there was 100 percent mortality in 24 hours in all groups and on the complement-fixation test all the antigens had approximately the same fixing ability.

In general, those viruses which had the shorter incubation period in swine also produced the most extensive primary lesions on the snouts and more secondary lesions on the feet. It was also observed that the more active pigs, as shown by their fighting with other pigs and struggling when caught, were more apt to develop secondary lesions on the feet.

The amount of vesiculation did not bear a direct relationship to the neutralizing antibody titers of the convalescent swine. The serum-neutralization titers varied within each group of swine approximately the same amount as they varied between the groups, except for the Missouri group, which had a titer over a log dose lower than the other groups.

The Missouri virus was the only one of the 12 which had previously been adapted to guinea pigs, and it showed greater differences than any other virus. It produced
primary lesions on the snouts and secondary lesions on the feet of all four pigs in the group but had the lowest serum-neutralization titer of all the groups. In guinea pigs it produced vesicles on all the Indiana-type immune guinea pigs and its hyperimmune guinea pig serum was one of the two highest in complement-fixing antibodies.

No correlation could be determined between the serum-neutralization titer of convalescent swine sera and the complement-fixing abilities of the hyperimmune guinea pig sera of the different groups. The Jesup, Roper, and Concan groups had high serum-neutralization titers with low complement-fixing abilities, while, on the other hand, the Missouri group had the lowest serum-neutralization titer and was one of the two highest in complement-fixing ability. Only the Brantley group maintained the same relative positions on both tests.

With the closer inspection of swine, necessitated by the eradication of vesicular exanthema, it has been found that New Jersey-type vesicular stomatitis is epizootic in the Southeastern States. Natural infection in swine has been diagnosed in this area every year since 1952. One would be inclined to believe that a change in the characteristics of the virus, as compared with the virus of bovine or equine origin, would have been necessary to produce this recent prevalence of vesicular stomatitis in swine. However, this study would tend to prove there has been no change in the New Jersey-type virus characteristics that could be associated with the natural appearance of the disease in swine.

SUMMARY

Experimental details of studies with 12 New Jersey-type vesicular stomatitis viruses are reported. Eight were of swine origin from field outbreaks, and four were known laboratory viruses.

1. Virus remained viable after six years' storage in a dry-ice chest at \(-70^\circ\text{C}\).
2. Mature chickens were susceptible to all 12 viruses.
3. The four laboratory viruses appeared more pathogenic to Indiana-type immune guinea pigs and to eight-day-old embryonating chicken eggs than were the eight field samples of swine origin.
4. The Missouri virus, which had been kept active by many animal passages, showed greater variations than any of the other samples tested.
5. The immunological variations among 12 virus samples were not of sufficient magnitude to permit separation into strains.
6. This study tends to prove there has been no change in the characteristics and serological properties of the New Jersey-type vesicular stomatitis virus found in natural outbreaks in swine from the virus previously found in cattle and horses.

ACKNOWLEDGMENTS

The writers are pleased to acknowledge the valuable advice of Dr. L. O. Mott of the Animal Disease and Parasite Research Branch, which was used in the studies here reported.

Acknowledgment is also made to Dr. E. W. Jenney of the Animal Disease Eradication Branch for assistance given in the serological portion of this work.
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2. Mott, L. O.: Epizootiology of Vesicular Exanthema of Swine. (Paper given at the
   Plum Island Symposium, Plum Island, New York, on Sept. 27, 1956.)
   Swine and Its Differential Diagnosis from Vesicular Exanthema and Foot-and-
   43-44.
7. Traub, E., and Mohlman, H.: Type Determination of FMD Virus by Way of the
   Complement Fixation Test. Part I, Experiments with Guinea Pig Sera and
8. Traub, E., and Mohlman, H.: Type Determination of FMD Virus by Way of the
   Complement Fixation Test. Part II, Experiments with Guinea Pig Serum and
The mandatory cooking of garbage before feeding it to swine was one of the most important measures in the programs proposed for controlling vesicular exanthema (VE) in California (1). Figure 1 illustrates the steadily declining incidence of vesicular exanthema as more and more garbage-feeding operators in California installed garbage-cooking facilities. During the latter part of 1954, however, outbreaks were still occurring on several ranches where cooked garbage was fed. In some instances and as far as it could be determined, the garbage was apparently cooked properly. One ranch that had experienced such recurrences was located in Alameda County of California, not too far from the University, facilitating a more detailed study. A portion of this study was reported on at the United States Livestock Sanitary Association meeting in New Orleans in 1955 (2). The results clearly demonstrated the occurrence of two outbreaks of VE in a herd within approximately 40 days. Although two distinct types of virus were recognized, the immunological types involved were not identified. This paper deals with three strains of VEV, including the two mentioned above, which were isolated from the same premises. The relative avirulence of the isolates may emphasize the need for revising some of our views of this infection in the event of its recurrence.

**HISTORY**

Since the study of the epizootiology of vesicular exanthema, including the identification of the immunotypes involved, began in 1951, at least eight outbreaks of the disease were recorded on this ranch since September 23, 1952 (Table I). It is evident that the two outbreaks that occurred in 1952 were due to two distinct immunological types of the virus. Although no outbreaks were reported for approximately 18 months (October 18, 1952, through May 22, 1954), at least six were observed during the succeeding 18 months. One strain of the virus was isolated from tissues of asymptomatic hogs submitted to slaughter 84 days after the December 29, 1954, outbreak. Because of the complexity of the types of virus involved, identification of each type has not been completed; but the data do show that the outbreaks are not all caused by the same immunological type. It is also interesting to observe that the types isolated defied a
NEW TYPES OF VESICULAR EXANTHEMA VIRUS

CORRELATION BETWEEN INCIDENCE OF V.E. IN CALIFORNIA IN 1954 AND 1955 AND COOKING GARBAGE BEFORE FEEDING TO SWINE.

Fig. 1

rapid identification and that isolates with these characteristics were made before garbage had been cooked.

MATERIALS AND METHODS

Isolation Techniques

To maintain strict isolation of the agents, all experiments were conducted in a barn and in laboratories that were specifically designed and used solely for the studies of VE. Personnel entering the building were required to remove all street clothing and change into coveralls, boots, and rubber gloves. The latter, after removal, were steam sterilized before reusing. Every effort was made to confine the studies to one immunological type of the virus in the barn at one time. However, in cross-immunity studies, which required the use of more than one strain, no other investigations were conducted at the same time, and investigators did not examine more than one group of animals without showering and having a complete change of clothing. Personnel leaving the building were required to shower before putting on their street clothing.

The investigator examined and cared for the hogs once each day to reduce chances of cross-infection. When each experiment was ended, all animals were killed within the unit, and the carcasses and refuse were sterilized for 24 hours at 15 pounds pressure before being removed from the building. The pens were thoroughly cleaned and washed down with a 2 percent lye solution.
### TABLE I

**History of Outbreaks of VE on a Hog Ranch Feeding Garbage**

<table>
<thead>
<tr>
<th>Outbreak No.</th>
<th>Date</th>
<th>Field Sample No.</th>
<th>Identification or Type</th>
<th>Garbage Fed</th>
<th>Number of Hogs</th>
<th>Percent of Hogs Involved</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23 Sept. '52</td>
<td>49</td>
<td>Not tested</td>
<td>Raw</td>
<td>6,000</td>
<td>20</td>
<td>Mild outbreak</td>
</tr>
<tr>
<td>2</td>
<td>18 Oct. '52</td>
<td>59</td>
<td>C</td>
<td>Raw</td>
<td>6,000</td>
<td>80</td>
<td>Severe outbreak. Numerous hogs with old snout and foot erosions from previous outbreak above.</td>
</tr>
<tr>
<td>3</td>
<td>22 May '54</td>
<td>299</td>
<td>CF-sl. fix with type D and E sera</td>
<td>Raw</td>
<td>5,000</td>
<td>50</td>
<td>Moderate outbreak</td>
</tr>
<tr>
<td>4</td>
<td>8 Sept. '54</td>
<td>310</td>
<td>Not tested</td>
<td>Raw</td>
<td>5,000</td>
<td>25</td>
<td>Many hogs observed with no visible lesions but off feed first few days of break. Fast recovery</td>
</tr>
<tr>
<td>5</td>
<td>29 Dec. '54</td>
<td>315</td>
<td>Hogs immune to 7 types and strain GF No. 1 Susceptible to Agent</td>
<td>Raw</td>
<td>4,500</td>
<td>10</td>
<td>All pens involved simultaneously. Had not completed arrangements for cooking prior to outbreak</td>
</tr>
<tr>
<td>6</td>
<td>23 May '55</td>
<td>—</td>
<td>GF No. 1</td>
<td>Cooked</td>
<td>4,500</td>
<td>?</td>
<td>No outbreak reported. Isolate from hogs submitted for slaughter</td>
</tr>
<tr>
<td>7</td>
<td>12 Sept. '55</td>
<td>330</td>
<td>F</td>
<td>Cooked</td>
<td>60</td>
<td>32.0</td>
<td>Experimental group introduced on premises</td>
</tr>
<tr>
<td>8</td>
<td>10 Oct. '55</td>
<td>332</td>
<td>G</td>
<td>Cooked</td>
<td>60</td>
<td>47.1</td>
<td>Experimental group introduced on premises</td>
</tr>
</tbody>
</table>
Experimental Susceptible Hogs

All animals used in the experiment were purchased as 30 to 50 pound pigs from grain-feeding hog ranches in California. The experimental animals were held in a quarantine shed located about 200 feet from the enclosed barn in which experiments with the agents were carried out. No immunization procedures were applied to the susceptible herd.

Recovered or Hyperimmunized Swine

Hogs recovered from VE that were used for cross-immunity studies were isolated for at least three weeks following initial infection. Hogs that were hyperimmunized for production of immune serum for the complement fixation test or for challenge purposes were held in the isolation barn until hyperimmunization was completed. Whenever possible, four intramuscular inoculations were given at four-day intervals, but when time or facilities did not permit fewer injections were administered. Hyperimmunization consisted of injecting 2 ml. of a five percent suspension of ground, freshly harvested vesicular coverings from the snout or feet of infected hogs. At least 10 days after the last injection, the animals were washed with four percent sodium carbonate and the pens with two percent lye solution. The washings were done at least twice at two-day intervals before removing the animals to isolation pens outside of the building within a fenced-in compound on the vesicular exanthema isolation grounds.

Virus

Type specific VEV for challenge purposes consisted of infected vesicular coverings of lesions of the snout and feet of hogs inoculated with the original strain-types A, B, C, D, E, F, and G, VE virus. Adequate supplies of virus were maintained by collections from infected swine vesicles at the height of their development. The vesicular coverings or granular material were scraped off with a curette and placed in a bottle containing glycerine phosphate buffer pH 7.4. The virus was stored at -20°C. until needed. When serial passages were made, the harvested coverings were immediately ground into a suspension for subinoculation. The virus suspensions used for inoculation were freshly prepared with a mortar and pestle as a 10 percent suspension by weight in buffered saline (pH 7.4). The suspension was centrifuged at 2000 rpm for 10 minutes and the supernatant fluid used as the inoculum.

Bacterial contaminants were found to produce lesions which, upon cursory inspection, were indistinguishable from a vesicle caused by a vesicular virüs. In a recent suspected VE outbreak the harvested vesicular-like coverings could not be successfully propagated by serial passage through hogs, but vesicular-like lesions were produced on the snout of hogs following intracutaneous injection of a suspension of the field material. The lesions consisted of a typical blanching and lifting of the epithelium about one to two mm. on either side of the tunnel. No extension of the lesion was observed, and serial passages of the coverings resulted in a loss of infectivity on the second or third passage. Bacteriological examination of the original inoculum revealed three types of colonies—two hemolytic strepto-
coccii and a diplococcus. Broth suspensions of individual colonies were prepared and when injected individually or in combination into the tarsal pads of guinea pigs, typical raised, blanched eruptions with extension of the lesions along the tunneled sites were produced within 48 hours. The lesions, if not carefully observed beyond their early development could be easily misinterpreted as a vesicular infection. By the 72nd and 96th hours, the covering was easily peeled; however, the contents consisted of a yellowish-to-greenish purulent exudate. Fig 2 shows a typical blanched and raised lesion on the left tarsal pad of a guinea pig 48 hours after an intracutaneous inoculation with the second serial passage of a pure culture of the three bacteria isolated from the suspected outbreak described above. The right pad shows the desquamated epithelium resulting from mechanical injury by the needle following injection of the same material which was treated with antibiotics. As a result of this observation, all inocula were treated with 5000 units of penicillin and 5000 micrograms of streptomycin per ml and allowed to stand in the refrigerator 30 to 60 minutes before use.

Fig. 2. Lesions on tarsal pads of a guinea pig produced with antibiotic treated (right) and untreated (left) suspensions of a mixture of 3 bacterial contaminants isolated from lesions on hogs with a suspected vesicular disease.
Before the experiments with the strains under study, no apparent difficulty has been experienced when 10 percent suspensions of known or unknown infectious material were inoculated intradermally or scarified into the dermal layers of the snout of susceptible swine. Experience with more recent isolates, particularly those described in this report, on the initial or in the first few passages, suggested that a more highly concentrated suspension or only the most susceptible sites should be used. An alternate method of inoculation consisted of preparing a paste of the infected tissues in a mortar with alundum and glycerine phosphate buffer (pH 7.4) containing the antibiotics. The inoculations were made by applying the paste with the pestle into the snout which was previously scarified with a scalpel. This method proved to be very effective.

EXPERIMENTAL RESULTS

Two new immunological types of VEV, designated as type \(F_a\) and \(G_a\), were isolated from a group of 60 recently introduced Eastern hogs, which were carefully inspected and observed at five to eight day intervals after arrival on the premises. Details of the experimental herd, which has been described, clearly demonstrated the occurrence of two outbreaks of the disease within the herd during a period of approximately 40 days, but the immunological types involved were not identified (2).

Identification of \(F_a\) (FS* 330)

At the peak of the first outbreak on September 12, 1955, typical vesicles from the snout and feet were harvested from four hogs. The material was immediately brought to the laboratory and susceptible hogs were inoculated on the same day. Inoculation of a 10 percent suspension intradermally into the snout of two hogs resulted in primary vesiculation by the 72nd hour. The lesions consisted of a slightly raised, blanched periphery, 2 to 3 mm. on either side of the lines of inoculation, with no appreciable extension or diffusion of the vesicles. No febrile response was recorded during the six-day observation period, but by the fifth day secondary lesions were found on the feet. A total of three rapid serial passages at three-day intervals through susceptible swine resulted in a slight increase in virulence. On the second serial passage maximum temperatures of 104.0° and 105.0° were recorded in two hogs inoculated, but vesicles appeared by the 48th hour with some degree of extension. Although the third serial passage in four hogs resulted in a temperature of 105.0° in one animal, maximum temperatures of not over 103.6°C. were recorded in the three penmates. By the 48th hour, however, the snouts of all four hogs presented typical blanched, raised vesicles, which spread until the entire snout was involved. Secondary vesiculation was found on the feet of all of the animals by the fourth or fifth days.

Purification of \(F_a\) (FS 330)

Table II shows the results of continued serial passage through hogs which had recovered or were hyperimmunized to each of types \(A_a\), \(B_a\), \(C_a\), \(D_a\), \(E_a\), and \(G_a\)

* FS = field sample.
TABLE I1

Serial Passage of FSS (FS 330) through Hogs Recovered or Hyperimmunized to Types A, B, C, D, E, and G (FS #330) VE Virus

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5th</td>
<td>2075</td>
<td>11/3/55</td>
<td>E—Rec. inoc. 34 da. prev. showing gen. VE</td>
<td>Harv. from hog immune to type E</td>
<td>106.8</td>
<td>+24</td>
</tr>
<tr>
<td>6th</td>
<td>2054</td>
<td>11/5/55</td>
<td>D—Hyper. 3X. 57 days</td>
<td>From 2075</td>
<td>105.0</td>
<td>+24</td>
</tr>
<tr>
<td>7th</td>
<td>2028</td>
<td>11/7/55</td>
<td>B—Hyper 3X. 91 days</td>
<td>From 2054</td>
<td>105.2</td>
<td>+48</td>
</tr>
<tr>
<td>8th</td>
<td>1759</td>
<td>11/10/55</td>
<td>C—Hyper 1X. 211 days</td>
<td>From 2028</td>
<td>103.6</td>
<td>+48</td>
</tr>
<tr>
<td>9th</td>
<td>1815</td>
<td>11/13/55</td>
<td>A—Hyper 1X. 193 days</td>
<td>From 1759</td>
<td>103.4</td>
<td>+48</td>
</tr>
<tr>
<td>10th</td>
<td>2063</td>
<td>11/22/55</td>
<td>G—Recovered. 40 days</td>
<td>From 1815</td>
<td>106.0</td>
<td>+24</td>
</tr>
<tr>
<td></td>
<td>2087</td>
<td>11/22/55</td>
<td>G—Recovered. 40 days</td>
<td>From 1815</td>
<td>106.4</td>
<td>+24</td>
</tr>
</tbody>
</table>

* Description of hogs (Rec.) recovered or (hyper) hyperimmunized 1 or more times, including the number of days last inoculation was administered.
† All inoculations made with a paste prepared with alundum and glycerine phosphate buffer (pH 7.4).

VE virus. The six serial passages were carried out within 19 days, and the virus was harvested and handled with care. Excellent symptoms in the form of fever reaching 105.0°C or more, with vesiculation within 24 to 48 hours, were noted in hogs immune to types E, D, B, and G, using the fifth, sixth, seventh, and 10th serial passages. The eighth and ninth serial passages (types C and A respectively) did not produce a febrile response, but secondary lesions were observed in the ninth passage. It is therefore evident that field sample #330 was apparently not a mixture as far as could be determined with the recognized immunological types of the virus available, and that the virulence of the virus appeared to vary even if carefully handled and passed without delay from hog to hog. The virus was not infective for guinea pigs when injected intracutaneously into the tarsal pads.

Table III shows the results of hogs recovered or hyperimmunized to FS #330 (F30) following challenge intradermally on the snout with each of types A, B, C, D, and G vesicular exanthema virus, indicating that the immunological difference is reciprocal. The virus was identified as type F30.

Identification and Purification of G (FS 330)

The occurrence of vesicular lesions in 47.1 percent of the hogs about four weeks after the first outbreak involved animals previously known to be infected with a
TABLE III
Results following Inoculation of Hogs Immune to Type F_{350} (FS 350) Inoculated with Types A, B, C, D, E, and G, VEV

<table>
<thead>
<tr>
<th>Hog No.</th>
<th>History of Hogs Recovered or Hypered w/330</th>
<th>Chal. with Type</th>
<th>Max. Daily Temp.</th>
<th>Results Lesions (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>2093</td>
<td>Hyper. Imm. 1X 92 days previously</td>
<td>A_{68}</td>
<td>106.4</td>
<td>+24</td>
</tr>
<tr>
<td>2109</td>
<td>Hyper 4X 51 days prev.</td>
<td>A_{68}</td>
<td>106.8</td>
<td>+48</td>
</tr>
<tr>
<td>2102</td>
<td>Hyper 1X 130 days prev.</td>
<td>B_{61}</td>
<td>104.4</td>
<td>+48</td>
</tr>
<tr>
<td>2125</td>
<td>Hyper 1X 130 days prev.</td>
<td>B_{61}</td>
<td>105.0</td>
<td>+48</td>
</tr>
<tr>
<td>2099</td>
<td>Hyper 1X 72 days prev.</td>
<td>C_{62}</td>
<td>104.6</td>
<td>+48</td>
</tr>
<tr>
<td>2139</td>
<td>Recover.—70 days prev.</td>
<td>C_{62}</td>
<td>103.8</td>
<td>+48</td>
</tr>
<tr>
<td>2372</td>
<td>Recover.—30 days prev.</td>
<td>D_{62}</td>
<td>105.8</td>
<td>+48</td>
</tr>
<tr>
<td>2373</td>
<td>Recover.—30 days prev.</td>
<td>D_{65}</td>
<td>106.6</td>
<td>+48</td>
</tr>
<tr>
<td>2450</td>
<td>Recover.—18 days prev.</td>
<td>E_{44}</td>
<td>105.6</td>
<td>+48</td>
</tr>
<tr>
<td>2476</td>
<td>Recover.—18 days prev.</td>
<td>E_{44}</td>
<td>106.6</td>
<td>+48</td>
</tr>
<tr>
<td>2597</td>
<td>Hyper 1X 9 days prev.</td>
<td>G_{65}</td>
<td>105.7</td>
<td>+48</td>
</tr>
<tr>
<td>2598</td>
<td>Hyper 1X 9 days prev.</td>
<td>G_{65}</td>
<td>104.0</td>
<td>+48</td>
</tr>
</tbody>
</table>

vesicular infection caused by another virus or another immunological type of VEV. The harvested vesicular lesions were prepared as a 10 percent suspension, treated with antibiotics, and injected intradermally into the snouts of five susceptible hogs and two swine which had recovered from the previous outbreak (type F_{350}). A febrile response (104°F to 106.4°F) was found in four of the seven inoculated hogs. The lesions, which appeared in all of the animals at the area of inoculation, developed by the 48th to the 72nd hour. They were characterized by a slight blanching 1 to 2 mm. along the lines of inoculation, accompanied with a minimum of elevation of tissue above the surface of the normal epithelium. The lesions progressed by extension but no typical vesicular development was found. The lesions were flat and dry, consisting of homogeneous grayish or a granular yellowish-to-brownish material covering an eroded substratum (Fig. 3). No diphasic febrile response or secondary lesions on the extremities were observed. The material of the first passage was removed with a curette and prepared as a paste with alundum and glycerine phosphate buffer and passed into five susceptible hogs. Similar results were obtained.

The yield from such poor lesions prohibited serological and cross-immunity studies. In an effort to increase the virulence and yield of virus the harvested infectious material from the second passage was serially passed through susceptible hogs five times within a 10-day period, using freshly developed lesions. Although this procedure produced scanty primary lesions, virulence increased slightly, and by
FIG. 3. Flat dry granular lesion on lower portion of snout 48 hours following inoculation of previously scarified area with a paste prepared with type G VeV. The darker area between the nostrils is scarified but not attacked by the virus.

the seventh serial passage typical vesicular lesions developed on the snouts of hogs 48 hours following inoculation with the paste on the previously scarified snout to yield a sufficient amount of virus for cross-immunity studies. Table IV shows the results of inoculating swine hyperimmunized or recovered from the six known immunological types of VeV. Primary vesiculation at the sites of inoculation developed in 48 hours in all but one instance in which primary vesicles developed in 24 hours (§2084). Febrile response to the virus and secondary lesions were not regularly observed either in hogs immune to the six types or in the animals known to be susceptible to VeV. The results definitely demonstrate the apparent low virulence of this strain even after seven rapid serial passages through susceptible hogs. Another series of serial passages of the virus through hogs immune to each of the six types of Ve demonstrated that the isolate was not a mixture of types. The virus failed to produce lesions in guinea pigs.

Swine recently recovered from FS 332 or hyperimmunized were found to be reciprocally susceptible to each of the six known immune types of VeV (Table V). The virus was designated as type GVeV.
### TABLE IV
Results of Inoculating Hogs Recovered or Hyperimmunized to Types A, B, C, D, E, and F, VEV with the 7th Serial Passage of Type G55 (FS 388)

<table>
<thead>
<tr>
<th>Hog No.</th>
<th>Immune Status* of Hogs</th>
<th>Max. Daily Temp.</th>
<th>Lesions (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td>2199</td>
<td>Susceptible</td>
<td>105.0</td>
<td>+48 None</td>
</tr>
<tr>
<td>2200</td>
<td>Susceptible</td>
<td>106.2</td>
<td>+48 +96</td>
</tr>
<tr>
<td>2201</td>
<td>Susceptible</td>
<td>103.0</td>
<td>+48 None</td>
</tr>
<tr>
<td>2159</td>
<td>A48 Hyper 1X 84 days prev.</td>
<td>106.2</td>
<td>+48 None</td>
</tr>
<tr>
<td>2162</td>
<td>A48 Hyper 1X 84 days prev.</td>
<td>106.4</td>
<td>+48 None</td>
</tr>
<tr>
<td>1870</td>
<td>B51 Hyper 3X 558 days prev.</td>
<td>103.2</td>
<td>+48 None</td>
</tr>
<tr>
<td>2023</td>
<td>B51 Hyper 3X 333 days prev.</td>
<td>103.8</td>
<td>+48 None</td>
</tr>
<tr>
<td>2153</td>
<td>C52 Hyper 1X 102 days prev.</td>
<td>104.6</td>
<td>+48 None</td>
</tr>
<tr>
<td>2156</td>
<td>C52 Hyper 1X 102 days prev.</td>
<td>105.8</td>
<td>+48 None</td>
</tr>
<tr>
<td>2046</td>
<td>D52 Hyper 3X 293 days prev.</td>
<td>103.8</td>
<td>+48 +120</td>
</tr>
<tr>
<td>2047</td>
<td>D52 Hyper 3X 293 days prev.</td>
<td>104.8</td>
<td>+48 +72</td>
</tr>
<tr>
<td>2068</td>
<td>E54 Recov. 280 days prev.</td>
<td>105.0</td>
<td>+48 None</td>
</tr>
<tr>
<td>2084</td>
<td>E54 Recov. 280 days prev.</td>
<td>102.0</td>
<td>+24 None</td>
</tr>
<tr>
<td>2098</td>
<td>F55 Hyper 1X 183 days prev.</td>
<td>103.0</td>
<td>+48 None</td>
</tr>
<tr>
<td>2105</td>
<td>F55 Hyper 1X 183 days prev.</td>
<td>105.0</td>
<td>+48 None</td>
</tr>
</tbody>
</table>

* See footnotes of Table II.

### Isolation of Strain GF1

Isolation of virus from tissues of asymptomatic hogs submitted for slaughter. Following the outbreak of December 29, 1954, (Table I) the ranch was placed under quarantine for 84 days. At the end of the quarantine period a permit was issued to transport 121 hogs to a plant for slaughter under the supervision of a USDA inspector. With the cooperation of the plant operator and the Federal Meat Inspection Division, arrangements were made to collect tissues from the carcasses for experimentation. No evidence of a VEV infection was observed on anti- and post-mortem inspections. It is estimated that the trimmings used in this study were obtained from the carcass approximately 30 minutes after the hogs were stuck and reached the inspection line. All equipment for this experiment was sterile, and trimmings of the various portions of the carcasses were placed in new and sterilized 30-gallon garbage cans. The tissues removed included longitudinal trimmings immediately off the center line of the brisket, which included some of the bone to obtain the marrow from approximately 20 to 25 hogs, uteri of two pregnant sows approximately ¾ term, and nongravid uteri of 48 hogs. Portions
TABLE V
Hogs Recovered or Hyperimmunized to Type G56 (FS 238) Challenged with Types A, B, C, D, E, and F, VE Virus

<table>
<thead>
<tr>
<th>Hog No.</th>
<th>Immune Status to Type G56*</th>
<th>Inoculum Type†</th>
<th>Max. Daily Temp.</th>
<th>Lesions (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>2164</td>
<td>Susceptible</td>
<td>A52</td>
<td>106.2</td>
<td>+24</td>
</tr>
<tr>
<td>2096</td>
<td>Hyper 3X 107 days prev.</td>
<td>A52</td>
<td>106.6</td>
<td>+24</td>
</tr>
<tr>
<td>2138</td>
<td>Recovered 91 days prev.</td>
<td>A52</td>
<td>105.0</td>
<td>+24</td>
</tr>
<tr>
<td>2176</td>
<td>Susceptible</td>
<td>B51</td>
<td>105.0</td>
<td>+48</td>
</tr>
<tr>
<td>2108</td>
<td>Hyper 3X 143 days</td>
<td>B51</td>
<td>106.0</td>
<td>+48</td>
</tr>
<tr>
<td>2123</td>
<td>Recovered 135 days</td>
<td>B51</td>
<td>104.6</td>
<td>+48</td>
</tr>
<tr>
<td>2156</td>
<td>Susceptible</td>
<td>C52</td>
<td>105.6</td>
<td>+48</td>
</tr>
<tr>
<td>2109</td>
<td>Hyper 3X 87 days</td>
<td>C52</td>
<td>105.6</td>
<td>+48</td>
</tr>
<tr>
<td>2124</td>
<td>Recovered 107 days</td>
<td>C52</td>
<td>105.4</td>
<td>+48</td>
</tr>
<tr>
<td>2148</td>
<td>Susceptible</td>
<td>D53</td>
<td>103.8</td>
<td>+72</td>
</tr>
<tr>
<td>2086</td>
<td>Hyper 3X 63 days</td>
<td>D53</td>
<td>105.6</td>
<td>+48</td>
</tr>
<tr>
<td>2126</td>
<td>Recovered 65 days</td>
<td>D53</td>
<td>105.0</td>
<td>+48</td>
</tr>
<tr>
<td>2143</td>
<td>Susceptible</td>
<td>E54</td>
<td>104.4</td>
<td>+48</td>
</tr>
<tr>
<td>2094</td>
<td>Hyper 3X 41 days prev.</td>
<td>E54</td>
<td>104.8</td>
<td>+48</td>
</tr>
<tr>
<td>2095</td>
<td>Hyper 3X 41 days prev.</td>
<td>E54</td>
<td>104.6</td>
<td>+48</td>
</tr>
<tr>
<td>2199</td>
<td>Susceptible</td>
<td>F55</td>
<td>105.0</td>
<td>+48</td>
</tr>
<tr>
<td>2087</td>
<td>Recovered 40 days</td>
<td>F55</td>
<td>106.0</td>
<td>+48</td>
</tr>
</tbody>
</table>

* See footnotes of Table II.
† 10% suspension of virus rubbed into scarified snout.

...of the heart, lungs, spleen, and liver were obtained from 30 hogs. The crural and renal lymph nodes, and particularly the nodes in the mesenteric fat, were collected from over half of the herd. The tissues were immediately brought back to the University and refrigerated at 4.0°C.

The next day the lymph glands and pieces of spleen, which were collected separately, were fed to 10 susceptible hogs that had been held without feed for the past 24 hours. During the succeeding two days, the hogs were fed the intact uteri as well as the bulk of the remaining tissues, consisting of spleen, mesenteric lymph nodes, and trimmings from the breast bone. Thereafter, the remaining tissues were fed until completely consumed by the sixth day.

On the fourth day after feeding, two hogs, S1961 and S1967, (Table VI) presented a febrile response, which was followed in 24 hours by typical vesicular lesions on the snout and the feet. All but two of the hogs presented typical vesicular lesions during the following six days. Over 1 gm. of vesicular material harvested...
NEW TYPES OF VESICULAR EXANTHEMA VIRUS

TABLE VI
Results of Feeding Tissues and Trimmings of Hogs Released from a VE Infected Premise for Slaughter following 84-Day Quarantine Period

<table>
<thead>
<tr>
<th>Hog No.</th>
<th>Results following Feeding of Tissues</th>
<th>Challenged with Immunotype VEV following Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DFF   Type</td>
</tr>
<tr>
<td>1960</td>
<td>T103.4°—Ves. lower lip 7th day</td>
<td>21 E (+)</td>
</tr>
<tr>
<td>1962</td>
<td>T103.0°—Ves. lower lip 7th day</td>
<td>21 E (+)</td>
</tr>
<tr>
<td>1967</td>
<td>T105.0°—T 4th day, Ves. ft. 5th day, lower lip 6th day</td>
<td>21 E (+)</td>
</tr>
<tr>
<td>1969</td>
<td>T104.2°—Negative</td>
<td>21 E (+)</td>
</tr>
<tr>
<td>1974</td>
<td>T105.6°—Ves. ft. and sn 7th day</td>
<td>21 E (+)</td>
</tr>
<tr>
<td>1961</td>
<td>T105.0°—T. 4th day, Ves. sn 5th day, Sec. T. rise 6th day, No. 2nd Ves.</td>
<td>20 D (+)</td>
</tr>
<tr>
<td>1963</td>
<td>T103.0°—Ves. tong and ft. 7th day</td>
<td>20 D (+)</td>
</tr>
<tr>
<td>1964</td>
<td>T103.8°—Ves. lower lip 10th day</td>
<td>20 D (+)</td>
</tr>
<tr>
<td>1965</td>
<td>T104.2°—Ves. sn 7th day</td>
<td>20 D (+)</td>
</tr>
<tr>
<td>1968</td>
<td>T104.0°—Negative</td>
<td>20 D (+)</td>
</tr>
</tbody>
</table>

T = Highest daily temperature recorded during 7 day observation period. Ves = vesicle. Ft = feet. tong. = tongue. sn = snout. DFF = Day following feeding. (+) = Vesicular lesions at point of inoculation. (+) = Primary and secondary lesions.

During the height of the disease from the two hogs first showing lesions when tested by the complement fixation test, indicated that the type of virus involved fixed complement in the presence of type D serum in low dilution with some slight cross-fixation with type E hyperimmune serum.

The hogs fed the trimmings and tissues obtained at the slaughter-house were divided into two groups following recovery and challenged with each of types Dₙ and Eₙ VEV three weeks after feeding, with types Bₙ and Cₙ 77 days after, and with type Aₙ 105 days after. All of the hogs that showed lesions were susceptible to the five known immunological types of VEV. A reciprocal immunity test, using hogs hyperimmunized to types Aₙ, Bₙ, Cₙ, Dₙ, and Eₙ VEV which were challenged with the virus isolated in the feeding experiment, suggested that a new immunological type or a virus of unusual characteristics may be involved.

The lack of adequate facilities prevented further study of this virus until the spring of 1956 when the virus was rapidly and serially passed six times through susceptible swine. Because of the mildness of the lesions that developed when the virus was first isolated, inoculations were made with a paste on the scarified snout. The first few passages resulted in the formation of dry, granular lesions at the points of inoculation. However, by the third serial passage, typical blanching,
followed by vesicular lesions with extension of the vesicles on the snout, was observed; secondary vesicles were formed on the extremities by the third or fourth day. The fifth and sixth passages resulted in a regular and typical pattern following inoculation of the snout, which included fever by the 24th hour accompanied or soon followed by typical blanching and vesiculation at the site of inoculation. Secondary lesions with or without a secondary febrile response occurred regularly. Table VII shows the results of the 7th through the 13th serial passages through hogs that had recovered from or were hyperimmunized to the seven immunologically distinct types of VEV (A, B, C, D, E, F and G). These results show that the hogs were readily susceptible to the new isolate and indicate homogeneity of type. The virus was designated, until more thoroughly studied, as strain GF 

| TABLE VII |
| Serial Passage of GF#1 Virus through Hogs Recovered or Hyperimmunized to Types A, B, C, D, E, F and G, VEV |

<table>
<thead>
<tr>
<th>Serial Passage No.</th>
<th>Hog No.</th>
<th>Date</th>
<th>Status of Principals*</th>
<th>Inoculum</th>
<th>Max. Daily Temp.</th>
<th>Results (Hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th</td>
<td>2599</td>
<td>4/9/56</td>
<td>F55 Hyper. 1X 112 days</td>
<td>Hog #2170. 6th Serial passage</td>
<td>105</td>
<td>+24 +72</td>
</tr>
<tr>
<td>8th</td>
<td>2187</td>
<td>9/4/56</td>
<td>G56 Recovered 49 days</td>
<td>Harvest of #2599</td>
<td>105.4</td>
<td>+24 +96</td>
</tr>
<tr>
<td></td>
<td>2188</td>
<td>9/4/56</td>
<td>G56 Recovered 49 days</td>
<td>Harvest of #2599</td>
<td>105.6</td>
<td>+24 +72</td>
</tr>
<tr>
<td></td>
<td>2192</td>
<td>9/4/56</td>
<td>G56 Hyper 2X 34 days</td>
<td>Harvest of #2599</td>
<td>104.6</td>
<td>+24 +96</td>
</tr>
<tr>
<td>9th</td>
<td>2157</td>
<td>9/7/56</td>
<td>A54 Hyper 1X 147 days</td>
<td>Harvest of 8th pass.</td>
<td>105.4</td>
<td>+24 Diphasic T. 72 hours. No secondaries +96</td>
</tr>
<tr>
<td>10th</td>
<td>2154</td>
<td>9/10/56</td>
<td>C54 Hyper 1X 168 days</td>
<td>Harvest of 9th pass.</td>
<td>106.4</td>
<td>+24 +72</td>
</tr>
<tr>
<td>11th</td>
<td>2175</td>
<td>9/13/56</td>
<td>B54 Hyper 1X 93 days</td>
<td>Harvest of 10th pass.</td>
<td>106.4</td>
<td>+24 +72</td>
</tr>
<tr>
<td>12th</td>
<td>2072</td>
<td>9/17/56</td>
<td>E54 Hyper 1X 10 days</td>
<td>Harvest of 11th pass.</td>
<td>105.6</td>
<td>+24 —</td>
</tr>
<tr>
<td>13th</td>
<td>2057</td>
<td>9/21/56</td>
<td>D54 Hyper 3X 369 days</td>
<td>Harvest of 12th pass.</td>
<td>105.4</td>
<td>+24 +72</td>
</tr>
<tr>
<td></td>
<td>2172</td>
<td>9/21/56</td>
<td>GF#1 Hyper</td>
<td>Harvest of 12th pass.</td>
<td>103.2</td>
<td>— —</td>
</tr>
</tbody>
</table>

* See footnotes of Table II.
Although the ranch under observation was invited by public sentiment and by the City and County Public Health authorities to vacate the premises as soon as the quarantine was lifted, further studies were desirable, and, at our request, the County Supervisors extended the time limit. It was desirable to determine whether the strains and types encountered may not show some peculiar characteristics of stability and persistence on the premises that may account for the recurrences of vesicular exanthema.

During the quarantine period there were approximately 1500 hogs on the premises, which were sold off in groups between November 1, 1955, and November 30, 1955.

Seven days after the last hog was removed and seven weeks after the peak of the last infection, 30 susceptible hogs were purchased from a grain-feeding ranch some distance from the implicated premises. The herd was taken directly to the ranch and released there. Six hogs, littermates of the 30 animals used for the contact experiment, were isolated and held at the University barn to be used as controls. Personnel of the Animal Disease Eradication Branch of the Agricultural Research Service were assigned to the experiment to insure that the animals were exposed to all feeding platforms, corrals, pens, mudholes, and runways. Since the premises were being dismantled, the animals had ample opportunity to come into contact with old as well as more recent dirt and debris which is often found in garbage-feeding establishments. Rolled barley for feed was purchased directly from a warehouse for this trial. The grain was thrown into various places where the infection might most likely be found, and the hogs were encouraged to eat and walk through these areas. Individual examinations—including inspection of the snout, mouth, feet, and body temperatures—were made daily for the first 12 days and thereafter on the 14th, 18th, and 25th days of the experiment. On all other days a critical herd inspection was conducted through the 31st day of exposure to the premises. One pig died of pneumonia on the 25th day.

At no time during the 31-day exposure period did any of the hogs exhibit clinical manifestations of VE. At the end of this period the hogs were brought to the University of California isolation barn on January 6, 1956, and divided into two groups. Group I, consisting of 14 hogs, was placed in a unit with three of the littermates that had until then been held in isolation as controls. Group II, consisting of 15 hogs, was placed in another unit with the remaining three control littermates for challenge with known VEV to detect whether the hogs suffered from a subclinical infection. As shown in Table VIII, all of the hogs in Group I were susceptible to types F, D, A, and B VE virus, and the hogs of Group II were found to be susceptible to types G, E, and C VE virus. The summarized data clearly indicate that hogs exposed to the premises for 31 days were all susceptible to challenge with the seven known immunotypes of VEV, but not in all instances to strain GF §1. Of the nine hogs of group I, which had been exposed to four immunotypes and had recovered from infection, only five were found to be susceptible to the virus and of the 13 hogs in group II, which had been exposed to three immunotypes and had recovered from infection, only six were susceptible to strain
### TABLE VIII

*Results of Challenging a Group of Thirty Pigs which had been Allowed Free Movement for One Month on Premises from which the Infected Herd Was Removed Seven Days Before*

<table>
<thead>
<tr>
<th>Strain GF No.1</th>
<th>Field exposed hogs</th>
<th>Susceptible litter mates</th>
<th>Virus controls</th>
<th>Virus controls immune to types A, B, &amp; D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Hogs</td>
<td>Type F₁₄</td>
<td>No. Hogs</td>
<td>Type D₂₄</td>
</tr>
<tr>
<td>GF No.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF No.1</td>
<td>14</td>
<td>6 T⁺ 6 T⁺</td>
<td>13</td>
<td>9 T⁺ 2 T⁺</td>
</tr>
<tr>
<td>Susceptible litter mates</td>
<td>3</td>
<td>1 T⁺ 2 T⁺</td>
<td>3</td>
<td>2 T⁺ 1 T⁺</td>
</tr>
<tr>
<td>Virus controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4 T⁺ 1 T⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1 T⁺ 1 T⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus controls immune to types A, B, &amp; D</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1</td>
<td>1 T⁺ 1 T⁺</td>
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</tr>
</tbody>
</table>

Group II

<table>
<thead>
<tr>
<th>Strain GF No.1</th>
<th>Field exposed hogs</th>
<th>Susceptible litter mates</th>
<th>Virus controls</th>
<th>Virus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Hogs</td>
<td>Type E₁₄</td>
<td>No. Hogs</td>
<td>Type E₂₄</td>
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<tr>
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<td>15</td>
<td>2 T⁺ 13 T⁺</td>
<td>15</td>
<td>12 T⁺ 1 T⁺</td>
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<tr>
<td>Susceptible litter mates</td>
<td>3</td>
<td>1 T⁺ 2 T⁺</td>
<td>3</td>
<td>3 T⁺ 3 T⁺</td>
</tr>
<tr>
<td>Virus controls</td>
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<td></td>
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</tr>
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</tr>
<tr>
<td>Virus controls</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

T = febrile response to 104°F. or above. + = Primary lesions at points of inoculation. ⊗ = Primary lesions followed by secondary lesions. 0 = no febrile response or lesions.
NEW TYPES OF VESICULAR EXANTHEMA VIRUS 317

GF #1. Although the number of susceptible controls for the virus did not equal the number of hogs in the field-exposed group, one of three hogs of the susceptible littermates of groups I and II, after exposure to the other immunotypes and recovery, was refractory to strain GF #1. Three susceptible experimental hogs obtained from other sources and not previously exposed to VEV were all susceptible to strain GF #1.

DISCUSSION

An investigation of the characteristics of VE virus strains isolated on a ranch on which the infection recurred even after the garbage fed to swine was cooked resulted in the recovery of at least two new immunologically distinct types of VE virus. The results of the study also showed that the strains recovered produced a milder form of the disease as compared with the strains and types of VE virus previously encountered. The isolation of types A, B, C, D, and E when first brought to the laboratory were mild on their first or second serial passage through susceptible swine; however, subsequent inoculations produced a moderate to severe disease depending upon the length of storage between passages. The low pathogenicity of these strains on their receipt in the laboratory may be explained by the storage of the field material under variable conditions by field veterinarians. All samples submitted to the laboratory for differentiation were held until the veterinarians were sure the outbreak was not foot-and-mouth disease. The latter was determined on the premises by inoculating several species of farm animals. Most samples were sent by common public carrier two to three weeks following the outbreak; however, others were delayed as long as six weeks. The new immunotypes, F, G, and particularly strain GF #1, were obtained from freshly harvested lesions. Attempts to cultivate the virus in a state of higher virulence by continuous transmission from one hog to another at rapid intervals resulted in a slightly increased pathogenicity. However, it was not possible to keep these strains at their higher virulence. It was observed that the slight increase in virulence induced by rapid serial passage was gradually lost when the virus was stored at freezing or refrigerator temperature for even a few days. It was customary therefore to make rapid serial passages through susceptible hogs each time before using the virus for experimental purposes. After intracutaneous injection of a 10 to 20 percent suspension into the snout, all three strains were characterized by their avirulence. The lesions in most cases were slow in developing and lacked invasiveness for attacking surrounding tissue; they were consequently small and circumscribed. The poor yields following inoculation by this method led to the use of a concentrated paste, which was prepared from the infectious material and applied by rubbing into the snout previously scarified with a scalpel. The lesions produced by these strains, whether the inoculations were made intracutaneously with a syringe or by scarification, were not typically vesicular. In most instances they consisted of a flat, dry, thin covering which upon manipulation or removal was found to be granular and grayish, brownish, or slightly yellowish. It may be possible that during the development of lesions caused by a virus of low invasiveness the thin, vesicular-like membrane rapidly degenerates into a granular mass. It is unlikely that bacterial contamination in the inoculum was the cause of these
tissue reactions, since all inocula were rendered bacteriologically sterile by previous treatment with antibiotics. Removal of the covering presented a glistening bright red or pink dermal substratum devoid of purulent exudate.

The febrile response prior to or during the development of primary lesions following inoculation under laboratory conditions was not always evident. During the field outbreak, when types F and G were harvested, body temperatures could not be taken (2). It was noticed, however, that the infection had little or no systemic effect upon the hogs. Their appetites were not affected and the animals were alert and active throughout the course of the disease. According to observations of hogs infected in the laboratory, the occurrence of fever in inoculated hogs was best characterized by its irregularity.

Secondary vesicles are not formed in all instances, with any of the strains of VE virus that has been studied to date. Vesicular stomatitis and even some strains of foot-and-mouth disease virus frequently fail to produce secondary vesicles (3, 4). The failure of the three strains of VEV to produce secondary vesicles was particularly obvious. Estimates of the percentage of generalization of the disease, as evidenced by the formation of vesicles at points other than the site of inoculation, were approximately 50 percent, 20 percent, and 10 percent following the inoculation of hogs with types F, G, and strain GF #1 respectively. A comparison of the activity of the three strains in hogs under laboratory conditions and of their pathogenicity in the field outbreaks from which they were isolated also revealed degrees of difference in virulence. Type G was the least pathogenic, type F appeared to be the most invasive, and strain GF #1 was intermediate in pathogenicity. Isolation of types of VE characterized by their low pathogenicity substantiates Doctor Crawford's observations in 1937 (5).

It is beyond the scope of this paper to discuss the possibility that the hogs from which strain GF #1 was isolated were "carriers." No lesions were observed on ante-mortem inspection, and because of the avirulence of the strain, which produced no obvious signs of the disease at the time of slaughter, it is entirely possible that the hogs were in the stage of incubation.

A notice to cease operations on the garbage-feeding ranch where these studies were conducted offered an opportunity to determine whether premises with a history of eight outbreaks of VE during the past three years harbored the virus. Exposure of 30 susceptible hogs for one month, beginning seven days after the herd was removed, presented no evidence of infection by indirect contact. No clinical symptoms of the disease were noted on a daily inspection, and the animals were susceptible to challenge with the seven known immunological types of the virus. Challenge of the hogs with strain GF #1 showed that only 50 percent of the farm-exposed hogs were susceptible. It cannot be ascertained whether the refractivity of the experimental group was due to the avirulence of the virus or perhaps in addition to a partial protection which may have been offered by the common antigenic components of the types of VE virus similar to that experienced with other viruses (6, 7, 12). The failure to demonstrate indirect-contact infection under field conditions soon after an infected herd had been removed substantiates the work conducted by Mott et al. under controlled laboratory conditions (8).

It is also interesting to observe that the last three isolates were made when the
garbage fed to the herd was cooked. Since only swine from the Midwest known to be free of the infection were being introduced and no infection was reported in the herd prior to their introduction, the source of infection must have had its origin on the premises. One can only speculate that inasmuch as the virus was not demonstrated on the ranch free of hogs, the infection must have been harbored in the previously exposed swine.

During studies of isolates from VE outbreaks in the field, which occurred between 1951 and 1954, it was observed that a disease due to a specific immunotype within an area occurred within a very definite pattern (2). Observations on this ranch showed that the outbreak in September 1952 was due to type C_s, which from the field veterinarian's report, differed from the outbreak 30 days previously inasmuch as the newly infected hogs also showed old lesions. Although no outbreaks were reported for the succeeding 18 months, at least six outbreaks were reported during the next 19 months. Two of the isolates (F_m and G_m) proved to be immunologically distinct from the five previously known types of VE. On preliminary study the four other isolates defied identification by cross-immunity studies into any of the seven known types. It is possible that mixtures of more than one type may be present; nevertheless, it is obvious that eight outbreaks have occurred on one ranch and half of the isolates studied were due to a distinct immunological type. It is possible that a genetic instability or mutation of one or more strains of the virus under field conditions may have been occurring. Dr. L. O. Mott proposed this possibility in his discussion of the epizootiology of vesicular exanthema at the Plum Island Symposium on Vesicular Diseases (9). His conclusions were based upon the failure to demonstrate the presence of type A_s VE virus in California since its occurrence in 1948, the multiplicity of types of VE virus, and the sudden and unexplained appearance of an immunologically distinct type of vesicular exanthema virus in the New Jersey outbreak of August 1955, after considerable evidence indicated that type B_s was the only type which escaped from California in 1952 (10, 11). The evidence produced through this study adds more circumstantial evidence that some change in the VE virus may be occurring, or that the origin of infection for susceptible hogs on a premise is introduced by some animal or other means not previously investigated.

CONCLUSIONS

1. Two new immunologically distinct types of VE virus were isolated from one group of hogs on a garbage-feeding ranch where at least eight outbreaks of the disease were reported during a three-year period. The sixth and seventh types were designated as F_m and G_m respectively.

2. A third strain of VEV, isolated from tissues of asymptomatic hogs submitted for slaughter from the same premises 84 days following a VE outbreak, has been described but not classified until more data is obtained.

3. All three strains of the virus were characterized by their low pathogenicity for hogs.

4. Thirty susceptible hogs allowed to root and roam for one month over the premises seven days after the infected herd had been removed showed no clinical
evidence of the disease and were susceptible to the seven known immunological types of vesicular exanthema virus.

5. The removal of exposed swine in the control of the disease and the possibility of mutation of the virus under field conditions are discussed.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Veterinarians and Livestock Inspector Agents of the Bureau of Animal Industry of the State of California and the United States Department of Agriculture for their conscientious efforts in collecting and submitting field samples and data which made this study possible.

Sincere appreciation is extended to Drs. W. A. MacDonald, Inspector-in-Charge, U.S.D.A., State of California, and W. L. Dicke, U.S.D.A., Supervisor, for their untiring cooperation, coordination and efforts which made the field studies possible.

Particular commendation is extended to Drs. C. E. Taylor (deceased), Supervisor, J. D. De Matte, Supervisor, E. V. Edmonds, C. J. Claire, and O. W. Sommer of the San Francisco district.

The technical assistance of Mr. Don Lundholm and Mr. Don Adams is gratefully acknowledged.

REFERENCES

The question of nutritive value of garbage as animal feed has had little technical attention. A report relating to garbage used for stock feed in England has been made available recently (1). In this country the refuse from various sources including street collections, hotels and restaurants, factories, military installations and various institutions constitute considerable volume, part of which is used for livestock, especially for swine feeding. In most of this material sanitary problems are of great importance. In recent years the practice of cooking has become widespread but raw garbage feeding still remains a problem to a degree in some states.

The study reported here resulted from efforts to establish sanitary control in certain areas in the State of New Jersey. Attempts were being made at hog feeding establishments to cook garbage in compliance with sanitary measures. Problems arose out of suspected effects on nutritive value ascribed to the use of high temperatures and prolonged cooking time. There were implications from certain disorders in hogs that nutritional factors could be involved.

The plan of this study was to evaluate the effects of cooking on nutritive value of garbage samples collected directly from the farms before and after being processed for feeding.

Samples were taken from the cooking equipment after agitation and immediately ground through a kitchen garbage disposal unit. Large bones and paper which this unit would not grind were removed from the sample prior to grinding. Representative samples were obtained of the raw garbage, then immediately after cooking to a minimum temperature of 160°F., and again after being held at this temperature over night. Collecting was repeated at each of the same farms of a second batch of garbage while raw, and again immediately after cooking to a minimum of 190°, and again after being held over night.

These samples were collected according to an original plan to obtain duplicate pint jars representing six treatments and four locations (or farms) for a total of 48 pint samples. They were immediately frozen and transported to Beltsville and stored in a freezer until the analytical work was done. Before the analyses were begun at Beltsville, one of the duplicates from farm No. 1 and six from farm No. 4 were used for completion of analytical work being done at the Virginia Experiment Station, leaving a total of 41 samples which were used for the analyses and feeding experiments given in this report.

Table 1 gives a comparison of the garbage samples based on the average protein content when the samples are classified according to heat treatment, time of sampling and original source. In Table 2 detailed analytical data are given for all of the 41 individual samples.

* Agricultural Research Service, United States Department of Agriculture. Credit is due Lena Struglia for the pantothenic acid determinations and W. R. Kauffman for the protein and dry matter values.
Comparison of Different Classes of Garbage by Average Protein Content

<table>
<thead>
<tr>
<th>Garbage and Treatment</th>
<th>Number of Samples</th>
<th>Average Protein Dry Basis per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heat treatments:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>14</td>
<td>19.16</td>
</tr>
<tr>
<td>Heated 160°</td>
<td>13</td>
<td>17.06</td>
</tr>
<tr>
<td>Heated 190°</td>
<td>14</td>
<td>18.48</td>
</tr>
<tr>
<td><strong>Time of sampling:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>14</td>
<td>19.16</td>
</tr>
<tr>
<td>Immediately after heating</td>
<td>14</td>
<td>18.80</td>
</tr>
<tr>
<td>Next morning after heating</td>
<td>13</td>
<td>17.64</td>
</tr>
<tr>
<td><strong>Source of garbage:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factory</td>
<td>11</td>
<td>27.08</td>
</tr>
<tr>
<td>City</td>
<td>12</td>
<td>15.13</td>
</tr>
<tr>
<td>Restaurant</td>
<td>18</td>
<td>15.10</td>
</tr>
</tbody>
</table>

The differences in protein content of the garbage samples, especially between the factory samples and those from the other sources indicate some difference in feeding value, which is related to original components rather than heat treatment. No effect on protein content due to heat treatment is indicated. The duplicate samples analyzed at the Virginia Experiment Station for crude protein and dry matter showed the same results.

**PANTOTHENIC ACID ASSAYS**

Pantothenic acid was determined by microbiological assay which included enzymatic digestion of the garbage samples. Considerable time was required to adapt methods to the special problems found with these particular samples. Further, the methods finally found satisfactory, were time consuming and after it was shown that reliable duplication could be obtained it was decided to save time by omitting determinations on some of the duplicate samples as indicated in Table 2. The pantothenic acid values obtained are given in the last column of this Table.

Observation of these values shows that pantothenic acid content, even of the raw garbage, when compared with the value established by the National Research Council for daily requirements of swine of 4.5 to 5 mg/lb of air dried feed, in most cases do not exceed this recommended level. Samples collected immediately after the garbage was heat treated to a minimum of 190°F for 30 minutes did not have any effects on the pantothenic acid content but when held over night did show some loss.

However, little or no loss occurred in the garbage held over night at 160°.

**RAT ASSAY FOR PROTEIN NUTRITIVE VALUE**

The amount of work and available resources made it advisable to composite samples from the six treatments for biological assay. That is, the six or seven samples available from each treatment were composited, thoroughly mixed and
### Table 2

**Results of Chemical Analysis and Pantothenic Acid Assay of Garbage from New Jersey Swine Farms**

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sample No.</th>
<th>Treatment</th>
<th>Temp.</th>
<th>Dry matter</th>
<th>Protein wet basis</th>
<th>Protein dry basis</th>
<th>Pantothenic acid—dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1 (Factory garbage)</td>
<td>A</td>
<td>Raw*</td>
<td>160</td>
<td>17.80</td>
<td>4.50</td>
<td>25.28</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Raw</td>
<td>160</td>
<td>18.33</td>
<td>4.86</td>
<td>26.51</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Cooked</td>
<td>160</td>
<td>17.29</td>
<td>4.28</td>
<td>24.75</td>
<td>5.12</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Cooked</td>
<td>160</td>
<td>17.00</td>
<td>4.54</td>
<td>26.71</td>
<td>—</td>
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<tr>
<td></td>
<td>A</td>
<td>Held</td>
<td>160</td>
<td>18.41</td>
<td>4.56</td>
<td>24.77</td>
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<td>Raw</td>
<td>190</td>
<td>17.99</td>
<td>5.08</td>
<td>28.24</td>
<td>3.71</td>
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<td></td>
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<td>190</td>
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<td>28.37</td>
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<td>190</td>
<td>16.67</td>
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<td>28.74</td>
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<td>28.92</td>
<td>1.46</td>
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<td>2.06</td>
<td>17.34</td>
<td>5.31</td>
</tr>
<tr>
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<td>B</td>
<td>Raw</td>
<td>160</td>
<td>12.04</td>
<td>2.12</td>
<td>17.61</td>
<td>—</td>
</tr>
<tr>
<td></td>
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<td>160</td>
<td>15.04</td>
<td>2.26</td>
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<td>Held</td>
<td>160</td>
<td>16.44</td>
<td>2.32</td>
<td>14.11</td>
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<td>Raw</td>
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<td>18.60</td>
<td>3.10</td>
<td>16.67</td>
<td>—</td>
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<tr>
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<td>A</td>
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<td>2.81</td>
<td>16.28</td>
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<td>12.28</td>
<td>2.35</td>
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<td>22.72</td>
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<td>4.50</td>
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<td>4.62</td>
<td>15.41</td>
<td>—</td>
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<td>160</td>
<td>26.36</td>
<td>3.70</td>
<td>14.04</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
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<td>Cooked</td>
<td>160</td>
<td>26.18</td>
<td>3.62</td>
<td>13.83</td>
<td>—</td>
</tr>
<tr>
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<td>4.44</td>
<td>15.62</td>
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</tr>
<tr>
<td></td>
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<td>Held</td>
<td>160</td>
<td>28.69</td>
<td>4.49</td>
<td>15.65</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Raw</td>
<td>190</td>
<td>25.11</td>
<td>3.68</td>
<td>14.66</td>
<td>4.28</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Raw</td>
<td>190</td>
<td>25.60</td>
<td>3.77</td>
<td>14.61</td>
<td>—</td>
</tr>
<tr>
<td></td>
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<td>190</td>
<td>20.04</td>
<td>2.84</td>
<td>14.17</td>
<td>5.86</td>
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<td>190</td>
<td>20.86</td>
<td>2.86</td>
<td>13.71</td>
<td>4.98</td>
</tr>
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<td></td>
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<td>Held</td>
<td>190</td>
<td>32.74</td>
<td>3.88</td>
<td>11.85</td>
<td>2.80</td>
</tr>
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<td>Held</td>
<td>190</td>
<td>33.85</td>
<td>4.00</td>
<td>11.82</td>
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<td>Raw</td>
<td>160</td>
<td>22.80</td>
<td>3.28</td>
<td>14.39</td>
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</tr>
<tr>
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<td>22.36</td>
<td>3.98</td>
<td>17.80</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Held</td>
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<td>18.47</td>
<td>2.89</td>
<td>15.65</td>
<td>3.49</td>
</tr>
<tr>
<td></td>
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<td>Raw</td>
<td>190</td>
<td>27.70</td>
<td>4.92</td>
<td>17.76</td>
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<tr>
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<td>4.29</td>
<td>18.13</td>
<td>5.08</td>
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<tr>
<td></td>
<td>A</td>
<td>Held</td>
<td>190</td>
<td>24.36</td>
<td>4.25</td>
<td>17.45</td>
<td>2.31</td>
</tr>
</tbody>
</table>

* The raw samples were not heated at all.
C. A. CABELL

TABLE 3

<table>
<thead>
<tr>
<th>Temperature</th>
<th>150°F (Immed. after)</th>
<th>160°F (Immed. after)</th>
<th>190°F (Next day)</th>
<th>190°F (Next day)</th>
<th>160°F (Raw)</th>
<th>Casein 120 mg day</th>
<th>Casein 160 mg day</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>7.0</td>
<td>7.3</td>
<td>7.9</td>
<td>8.0</td>
<td>9.1</td>
<td>13.1</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Least significant mean difference (LSD) = 3.9.

the protein nutritive value determined for the composited sample representing each treatment.

Limitation of the amount of sample available for feeding made it necessary to use the rat repletion method as described from this laboratory (2).

The samples were fed to rats on an equal nitrogen basis at a rate of 120 mg of nitrogen per rat per day and compared with a standard protein, casein fed at the same level and a higher level. Each sample was fed to 8 rats. Total consumption of the garbage samples was obtained as there was no waste or refused material. Mean weights of the animals are given in Table 3. These means are arranged in numerical order to show statistical significance as determined by Duncan's Range Test (3). There is no significant difference at the 5% level of probability in the italicized means. This shows that no significant difference was found in protein nutritive value in any of the garbage samples.

SUMMARY AND CONCLUSIONS

The effect of cooking temperatures on nutritive value of garbage being fed to swine in the New Jersey area at four different farms was studied.

Chemical analysis for protein content and dry matter and biological assays for protein nutritive value indicated no effects on these factors when the garbage was heated to a minimum temperature of 190°F for 30 minutes and held over night.

Heat treating of the garbage at 190°F for 30 minutes did not lower the percentage of pantothenic acid present, but when held over night, there was a loss in its content. Heating the garbage at 160°F and holding it over night did not produce any loss in any of the factors studied.

REFERENCES

THE DIFFERENTIATION OF VESICULAR DISEASES
BY SEROLOGICAL PROCEDURES

CHRISTINE E. RICE, M.A., Ph.D.*

The three major vesicular diseases affecting livestock, foot-and-mouth disease in cattle and swine, vesicular exanthema in swine, and vesicular stomatitis in horses, cattle, and swine, cannot be differentiated on the basis of clinical symptoms alone. Although inoculation of susceptible animal species was for many years the most important and universal diagnostic method, in many countries it now serves rather as a supplement and support to laboratory methods, being resorted to only if the vesicular material submitted is inadequate in amount or character for laboratory tests. Obviously, however, animal tests are always essential when it is suspected that the vesicular condition may represent an outbreak of foot-and-mouth disease or vesicular exanthema in an area or country previously free of the disease in question. With so much at stake, all available tests should be made to establish the correctness of such a diagnosis.

But it is with in vitro serological tests that I am dealing to-day, and more particularly with the complement-fixation test, not only because it is the method with which I am most familiar, but also because it is the in vitro serological test that has been utilized most widely in the study of these three diseases. The relatively extensive literature on its application in the differential diagnosis of foot-and-mouth disease and vesicular stomatitis has been reviewed in papers by Brooksby and others (1-13), that on the diagnosis of vesicular exanthema is described or referred to in the publications of Bankowski and his associates (14-16). The present paper will point out some of the difficulties that have been encountered in the development of these tests and how they have been overcome. It will confine itself to a discussion of some of the basic procedures rather than to the presentation of new experimental material.

DETECTION AND IDENTIFICATION OF VIRUS IN VESICULAR MATERIAL

Complement-fixation tests have been used for two general purposes, (a) for the detection of virus in vesicular material and the determination of its nature, and (b) for the demonstration of antibody in the serum of recovered animals. For the detection of virus, the vesicular material should be collected from the affected animal when the viral content may be expected to be at its maximum, usually at the peak of fever or slightly later when the vesicles on the tongue or lips are well developed and before exfoliation has occurred. Material from foot lesions may be utilized in vesicular exanthema but has not proved satisfactory in the case of foot-and-mouth disease. When sufficient in amount, the tests may be made directly with this field material. If limited in amount, or in poor condition, a susceptible animal should be inoculated and the vesicles harvested at the proper time. Tissue may be treated in various ways, the simplest being grinding in a

* Animal Pathology Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.
mortar with sand or alundum and buffer. The anticomplementary or non-specific properties may be reduced by differential centrifugation or extraction with lipid solvents.

These tissue extracts are then tested by a standard technique for complement-fixing activity with known, previously-standardized antisera produced in a susceptible species. The selection of a susceptible species for preparation of antisera avoids the complicating factor of antibodies for foreign proteins which develop when the viral inoculum is derived from another animal species. Absorption of these antibodies is difficult and seldom satisfactory.

Antisera for the six types of foot-and-mouth disease virus and for the two types of vesicular disease virus are produced by hyperimmunization of guinea pigs with strains adapted to this species. Hyperimmune sera are preferred for preliminary testing because of their broad reactivity with viral strains within types. For more detailed study of strain differences within types, convalescent sera of lower titer and narrower specificity, and more particularly antisera for the newly-isolated strain itself are required. This shift in the antigenic character of strains within types has probably created greater difficulties in vaccine production than it has in differential diagnosis and typing of strains.

Standard antisera for the detection of the various types of vesicular exanthema virus are derived from swine hyperimmunized with these respective types. In lower dilutions such antisera exhibit definite cross fixation between types, but in higher dilutions are type specific. As will be discussed in greater detail later, the so-called "procomplementary" activities of swine antisera which have constituted one of the major difficulties in the development of the test have now been overcome (14).

The complement-fixation techniques that have been adopted fall into two general categories, (a) the popular antigen-dilution form with constant antiserum and complement, and (b) a more precise test with constant antiserum and antigen and varying complement. In both forms of test, the indicator system has been sheep red cells sensitized with hemolysin prepared in the rabbit or horse, and guinea-pig complement. The antigen-dilution technique is simpler and reasonably satisfactory when the tissue extract under examination is of moderately high titer so that definite reactivity is demonstrable well beyond the anticomplementary or lytic range. In selecting the standard complement dosage an attempt has been made to adjust for variations in those properties by using a higher complement dose in testing foot-and-mouth disease viral antigens of equine or viral origin which are ordinarily somewhat anticomplementary, and a lower complement dosage in tests of antigens derived from swine tissue which tend to be hemolytic.

The more precise test with varying complement dosage developed by Brooksby (8) which resembles the New York State quantitative technique, avoids this arbitrary decision. Even though the test antigen be anticomplementary or lytic, its range of fixability may be expected to fall within the series of complement dilutions used. Consequently the similar or definitely greater degree of fixation in the presence of antiserum as compared with that in the antigen control without antiserum, permits evaluation of the reaction as negative or significant.
The selection of the time and temperature of fixation has also represented a compromise. Here, as in well recognized, longer periods of fixation in the cold yield higher titers but with a concomitant increase in non-specific fixation with tissue extractives. For diagnostic purposes, it has seemed preferable in certain laboratories, therefore, to sacrifice a certain degree of sensitivity in the interest of greater specificity and ease in the interpretation of the test result.

DETECTION OF ANTIBODY IN SERA

The conventional complement-fixation techniques for the detection of antibody were developed principally for testing human sera. When these methods were extended to sera of various animal species, difficulties were immediately encountered. Serological studies of vesicular diseases have constituted no exception. Although antibodies to other viruses have not always been easy to detect by complement-fixation methods in convalescent horse sera, those for the New Jersey and Indiana types of vesicular stomatitis may be readily demonstrated and differentiated in experimentally-infected horses. Unfortunately from the standpoint of epidemiological surveys, the titers decline rapidly. Serum dilution tests with constant amounts of standard antigen and constant or varying complement dosage may be used the latter depending upon the desired accuracy of the titer estimate.

When it came to testing bovine or swine sera by complement-fixation methods for evidence of antibodies for foot-and-mouth disease or vesicular stomatitis viruses, negative results were usually obtained even though the animals from which these sera were collected proved immune to challenge and mixtures of live virus and the serum in question proved non-infective or neutralized when injected in the bovine tongue or in baby mice. A somewhat greater percentage of the bovine antisera exhibit fixation if tested without inactivation but since many normal unheated bovine sera are anticomplementary or fix complement non-specifically with various tissue extracts, the introduction of such a modification into the test would be confusing from the diagnostic standpoint. Even a comparison of the relative complement-fixing activities of unheated acute phase and convalescent specimens from the same animal would not avoid this difficulty in interpretation since serial bleedings from the same animal may vary in non-specific properties particularly if a febrile period has intervened.

The negative results recorded with the majority of cattle sera, appear to result from a failure of the complex formed between antibody and homologous viral antigen to fix guinea-pig complement or from a reversible fixation, such that on the addition of the indicator system hemolysis of the sensitized sheep red cells can ensue. Limited kinetic studies offer some support to the latter hypothesis. One method of overcoming this difficulty which I personally have found useful, is through application of the indirect complement-fixation test developed for the detection of non-complement-fixing antibody in chicken antisera (11). In this test the bovine sera are mixed with the standard viral antigen and after a suitable period for combination, complement and the corresponding guinea-pig antiserum are added. If on the addition of the indicator system hemolysis occurs, it denotes that the reactive groups on the antigen have combined with the bovine antibody.
leaving none available to combine with the guinea-pig antiserum to form complement-fixing complexes. On the other hand if no hemolysis is observed, it indicates that little or no antibody was present in the bovine serum, leaving the antigen free to combine with the guinea-pig antiserum with resultant fixation of complement.

Other workers have developed several other *in vitro* techniques for the titration of antibody in the sera of cattle recovered from foot-and-mouth disease. A method of salt treatment which enables the use of unheated bovine serum in complement-fixation tests has been described by Serra and Guarine (17) and found satisfactory by Nordberg and Schjørene-Thiesen (18) but less so by other investigators. A combined neutralization and complement-fixation test was developed by Brooksby and Wardle (19), an ingenious test which has been taken up in several other laboratories (20). This test involves the inoculation of tissue cultures of bovine epithelium with mixtures of bovine serum and foot-and-mouth disease virus and the titration of the culture after a suitable period for virus propagation, for complement-fixing activity with guinea-pig antiserum of the corresponding type. A hemolytic test with horse complement and guinea-pig erythrocytes sensitized with heated bovine serum has recently been described by Dinopoullos *et al.* (21) which appears promising for the titration of vesicular stomatitis viral antisera of bovine origin.

The difficulties encountered in developing satisfactory complement-fixation tests for the titration of swine sera have been of a different nature. Although swine antisera contain antibodies capable of fixing guinea-pig complement with homologous viral antigens, these cannot be readily detected because of the presence of "procomplementary" substances. These substances which greatly augment the hemolytic activity of guinea-pig complement are present in normal as well as immune swine sera. When swine serum is added to other complement-fixing systems as for example to a mixture of an antibacterial rabbit antiserum and homologous bacterial suspension, the fixation of complement is markedly inhibited, its effect being the greater the earlier it is added. Because of the known high content of swine serum in third component of complement, it was suspected that this component is the substance involved. However, other evidence would suggest that this surmise may not be correct or at least afford only a partial explanation. In any case, the practical difficulty has been overcome in two ways.

The first alternative adapted by Bankowski and his associates (14,15) has been to accept the existence of these interfering substances and devise methods of adjusting to their presence. In the method they evolved, the amount of complement in the test is reduced to allow for the procomplementary activity of the serum. This has been done by titrating the complement in the presence of swine serum and estimating the unitage to be used in the test accordingly. This method while useful for antigen titration, is not so practical for serum titration, the higher dilutions showing decreasing procomplementary activities. A varying complement method with suitable serum controls has been investigated in our laboratory with a fair measure of success although the results are difficult to express numerically.

The other alternative adapted by Doctor Boulanger in our Institute was to devise methods of removing or inactivating the "procomplementary" substance. Cer-
tions to initial evidence suggested that it might be lipoprotein in nature. Of the several methods of treatment attempted, alcohol or ether extraction in the cold were moderately successful, but adjustment of the pH to approximately 4.2 and re-adjustment after standing overnight in the refrigerator proved the simplest and most satisfactory. After such treatment normal swine sera no longer augment the hemolytic activity of complement nor do they reduce the fixation by rabbit antibody-antigen systems. Similarly after pH adjustment, complement-fixing activity with vesicular stomatitis virus antigens is readily demonstrable in heated sera of swine convalescent from this infection. Furthermore, the fixation is type specific. Since the development of the method, we have fortunately not had an opportunity in Canada to apply the method in the detection of antibody for foot-and-mouth disease or vesicular exanthema viruses.

If complement-fixation tests are applied in epidemiological studies of vesicular stomatitis viral infection in the wild life population, no doubt other difficulties may appear but it is hoped that the experience with swine and bovine sera will alert all investigators to the possibility that negative results in standard complement-fixation tests do not necessarily indicate the absence of antibody.

SUMMARY

Some of the problems that have arisen in relation to the in vitro differentiation of vesicular diseases in cattle, horses and swine have been described, with particular emphasis on the complement-fixation test. Certain of the general principles underlying the satisfactory performance of such tests and the modifications introduced have been discussed.

For the detection by complement-fixation tests of a particular virus in suspected vesicular material, standard antisera for the six types of foot-and-mouth disease virus, the two types of vesicular stomatitis virus and if possible for the five or more types of vesicular exanthema virus should be maintained in the laboratory. Where permissible, corresponding standard control antigens or material from which they can be prepared should be available to check the reliability of the test.

For the detection of antibodies for any one of these viruses in convalescent bovine, porcine or equine sera, corresponding standard viral antigens are required. This limits, the performance of such tests to laboratories where such antigens can be maintained without hazard to the livestock population of the country concerned.

REFERENCES


The Animal Disease Eradication Branch reports that sixty-five outbreaks of suspected vesicular conditions were investigated during the first eleven months of this calendar year. To date, twenty-four of these sixty-five outbreaks have been identified as vesicular stomatitis, New Jersey type; one Indiana type; and three outbreaks as vesicular exanthema.

VESICULAR STOMATITIS (VS)

The states in which investigations were carried out, and in which vesicular stomatitis was diagnosed, were North Carolina, South Carolina, Georgia, and New Mexico. The New Jersey type was reported in four states; one case of Indiana type was reported in New Mexico.

It is the general consensus of regulatory officials conducting the work in those areas where vesicular conditions were reported, that the disease is present enzootically. The more they investigate, the more they are able to uncover. One can only conclude that the disease has actually been more widespread in these, and other states, than is indicated by reports. Failing to detect or ignoring occurrences of vesicular stomatitis is entirely inconsistent with the all-out efforts being directed toward eradication of vesicular exanthema or the stamping-out program that would be invoked if foot-and-mouth disease were diagnosed. Your committee cannot avoid a critical attitude regarding reporting of any and all suspected vesicular diseases, including vesicular stomatitis. Diagnosis is essential.

Epizootiological studies presently conducted by the University of Wisconsin in cooperation with the United States Department of Agriculture, Agricultural Research Service, and reported on here today by Doctors Hanson and Karstad, should be very beneficial in discovering some of the ways in which vesicular stomatitis is perpetuated. If we can determine the actual incidence of the disease, and possible reservoir in which it is harbored, then we may be able to take control measures that will suppress the condition and favor eventual eradication.

The confirmed outbreak of vesicular stomatitis, Indiana type, is the first of this type since 1942. Previous to the 1942 outbreak, a case was identified in 1927.

An epizootiological study on this year’s new outbreak in New Mexico revealed that the condition may have been present there for some time, but was not reported. Numerous comments were received from natives in the area that the horses have been infected with this condition from time to time for many years. This is a very disturbing situation.

The committee urges that vesicular stomatitis be declared a reportable disease,
and that a herd in which it is present be placed under quarantine for at least thirty days after recovery of the affected animals. The present lack of such procedures emphasizes a very serious problem that exists within the United States. We are confronted with a condition in cattle and swine that cannot be distinguished clinically from foot-and-mouth disease. Evidently numerous cases of vesicular stomatitis are not reported and are not quarantined. This situation is ideal for allowing foot-and-mouth disease to become well established before we know that it exists in the country.

**VESICULAR EXANTHEMA (VE)**

Three cases of vesicular exanthema, confined to an isolated area, were reported in the United States, in New Jersey, to date this calendar year. There were about 9,500 swine involved. The swine in the initial outbreak were disposed of. Negotiations between Federal and State officials, and the owners of the last two premises, are being carried out to dispose of the swine as soon as possible.

It is urged that prompt action be taken by Federal and State agencies to dispose of these hogs. Based on experiences in California, it is probable that the disease will persist indefinitely unless complete depopulation takes place.

There is evidence that new immunological types of VE virus are occurring which appear to be due to mutation of the parent strains, further emphasizing the need to eliminate these reservoirs of infection.

The last previous outbreak was reported on November 10, 1955, in Santa Clara County, California.

California now has not experienced an outbreak for more than a year, the longest period during which the state has been free from the disease since 1939—over seventeen years. Since late in 1939, the cycle of the disease has followed high and low peaks about every three years. Following the usual cycle, the year 1956 ordinarily would have been one of high incidence—instead, there have been no new cases to date. Although it is too soon to state positively, the apparent break in the cycle is due to the following factors:

1. Quarantine restrictions on the movement of infected and exposed swine, and swine and pork produced on raw garbage;
2. Cooking of all garbage fed to swine;
3. Attention to management and sanitation factors;
4. Final destruction of the remaining infected and exposed swine on February 8, 1956, thus removing the last known vestiges of the virus in California.

There is strong evidence available in California that the United States should never return to the practice of feeding raw garbage.

Progress has been made in Texas relative to initiating an inspection program. Agricultural Research Service VE inspectors were sent there from several states to conduct a survey and to determine the need for inspections of garbage feeding premises. Personnel for the purposes of carrying out these inspections now are being employed.

New Jersey and Connecticut as yet do not have garbage cooking laws. A strong attempt, furthermore, was made in Massachusetts to repeal that state's garbage cooking law. This is the first indication of a backward trend in the national VE program since its initiation.
Complacency has been encouraged by the fact that the incidence of VE has been greatly reduced. In some cases, violators of garbage cooking laws are not being prosecuted, because state officials apparently no longer consider the laws of vital importance; and in a few cases, judges or magistrates have set aside the cases of violation.

The increasingly apparent success of efforts to eradicate VE are most heartening. The warning against complacency by the 1955 committee is worthy of repetition at this time. It is especially desirable that, in addition to repeated routine inspection of garbage feeding premises, every effort be made to continue effective cooking. Doctor R. A. Bankowski's report here today on the finding of new immunological types, having a low pathogenicity and ability to produce a high rate of sub-clinical infection, further emphasizes the importance of continuing all precautionary measures, such as cooking of garbage and regular careful inspections, which should aid in preventing the disease from gaining another foothold. Even if VE were to be completely eradicated, the transmission of such diseases as hog cholera, trichiniasis and other vesicular diseases through unprocessed garbage, in themselves merit continuing improvement of cooking procedures and the general application of the most effective processes that may be devised.

The Committee strongly recommends that each state official bring to the immediate attention of the livestock industry in his state the need for continuing this very vital precautionary measure against introduction and spread of swine diseases.

**INCIDENCE OF FOOT-AND-MOUTH DISEASE (FMD)**

Rinderpest or foot-and-mouth disease have been determined to exist in most of the livestock-producing countries of the world except: Australia, New Zealand, Norway, the Channel Islands, Republic of Ireland, Northern Ireland, Canada, the United States of America, Mexico, Central America, and the islands of the Caribbean except Martinique. There has been no change in the past year in this determination of countries infected with one or the other of these diseases. The incidence of foot-and-mouth disease in Europe has increased somewhat in 1956. The Office of International Epizooties reported simultaneous outbreaks in different places, often great distances from each other, and that these outbreaks were caused by different types of viruses; in Germany for example, C, O, A, A, and O; in France and Italy, types O, A, and C.

A new series of outbreaks began in Great Britain during the early part of August, some due to type C, and some due to type O. From January 1 to September 15, 1956, there have been ninety-nine outbreaks in Great Britain; 18,351 animals were slaughtered as diseased or exposed to infection, compared with six outbreaks and 956 animals slaughtered during the same period of 1955. At this time, the disease has been confined to limited areas, and the extent of the outbreak continues to be considerably less than during the 1951–52 epizootic.

There were no outbreaks in Germany during April and May, but many new centers have recently reappeared, and control measures, using vaccination, are being carried out.

Recent reports have been received from Peru concerning several outbreaks,
principally in the Lima area. In Venezuela, although the Ministry of Agriculture reported no incidence during 1955, outbreaks began to recur in January, 1956.

An outbreak in Switzerland originated on May 19 from a transit shipment of Belgian hogs to Italy. It involved 101 farms, 1,145 cattle, 884 hogs, as well as a few goats and sheep. This outbreak was eradicated by July 7. Another outbreak occurred July 9 near the Italian border. There have been several small outbreaks since.

Despite a relatively low incidence in cattle, outbreaks of the disease in swine in the Netherlands have been reported recently. Whether or not this is a truly swine-adapted virus has not been determined, according to last reports.

**RESEARCH DEVELOPMENTS**

Studies at the Research Institute at Pirbright, England, have revealed the presence of two differently sized particles in suspensions of FMD virus, a larger infective particle and the considerably smaller complement-fixing particle. Similar studies at Pirbright have disclosed a number of apparently distinct, variously sized particles in suspensions of VS virus. From Pirbright we also learned that a seventh type of FMD virus, known as Asia I, has been found in material received from Asia.

In addition to previously reported work in propagation studies of FMD virus at Plum Island, virus of high titer has been produced in suspensions of surviving kidney cells of several species of animals. Preliminary trials indicate practicability of this method of producing quantities of virus. Preliminary comparative inoculations of cattle of different ages and breeding have revealed, to date, no material difference in susceptibility. Repeated exacting tests to determine the thermal death point of FMD virus have demonstrated that under some conditions the virus in minimal quantity may survive exposure to temperatures as high as 80° C. for as long as six hours, when the heated suspension is injected in large quantities into cattle.

Current studies are being made on the propagation of vesicular exanthema virus in tissue culture at the Animal Disease and Parasite Branch of the Agricultural Research Service (Beltsville, Maryland) and the University of California at the Naval Biological Laboratory (Berkeley), and in the School of Veterinary Medicine (Davis). It is hoped that this technique will provide a versatile tool for cultivation, diagnosis and assay of this host-specific virus.

Unreported cooperative studies, carried out on the Island of Lindholm in Denmark by an American worker, have led to the development of a technic whereby antibodies against FMD virus may be demonstrated by direct complement-fixation in convalescent or vaccinated cattle. The test promises to be an effective tool for diagnosis and epizootiological studies.

Construction of the new laboratory on Plum Island has not progressed as rapidly as expected, although the facilities were dedicated September 26. Months of testing and checking will still be required before studies of FMD may be expanded in the new building. Research is expected to begin in the new laboratory shortly after the first of the next calendar year.
REPORT OF COMMITTEE ON NOMINATIONS


The Report of the Committee on Nominations, of which Dr. C. E. Kord of Nashville, Tennessee is Chairman.

Dr. R. L. West: Mr. President, it was necessary for Doctor Kord to leave, and he requested me to present his report.

Your Committee on Nominations presents the following names and recommends their election to the offices designated for the coming year:

President—Dr. G. H. Good, Wyoming.
First Vice President—Dr. J. Milligan, Alabama.
Second Vice President—Dr. H. U. Garrett, Iowa.
Third Vice President—Mr. F. G. Buzzell, Maine.

President Brueckner: Thank you, Dr. West.

The Chair is ready to receive nominations from the floor for the office of President.

Dr. T. C. Green: Mr. President, I move that nominations be closed for all offices.

[The motion was severally seconded, was put to a vote, and was carried unanimously.]

Dr. R. W. Smith: Mr. President, I move that the Secretary cast one ballot for the entire slate, and that that ballot be the vote of this General Assembly.

Dr. A. K. Kuttler: Second the motion.

[The motion was put to a vote and was carried unanimously.]

Secretary Hendershott: According to your instructions, it is my pleasure to cast the unanimous ballot of this Association in favor of Dr. George H. Good, Wyoming, for President; Dr. John A. Milligan, Alabama, for First Vice President; Dr. H. U. Garrett, Iowa, for Second Vice President, and Mr. F. G. Buzzell, Maine, for Third Vice President.

President Brueckner: Will Doctor Peterson escort Doctor Good to the rostrum, please? Doctor Sugg, will you please escort Doctor Milligan, and Doctor Bendix, will you escort Doctor Garrett? Doctor Canty, bring up Francis Buzzell, please.

Doctor Good, it is a pleasure for me to turn over to you the responsibility and pleasure of the office of President for the year 1956-1957.

Doctor Good: Thank you, Doctor Brueckner. I greatly appreciate being elected to the Presidency of this Association, and with the able assistance of our good Secretary and other officers, committees and members, I am sure we will have another successful year in 1957.

Thank you. [Applause]

President Brueckner: Dr. J. Milligan, it is a pleasure for me to receive you as the new First Vice President of the United States Livestock Sanitary
Association. It will give you an opportunity to get more closely in touch with the affairs of the Association in this new office.

DR. J. MILLIGAN: Doctor Brueckner and members of the Association, I am indeed grateful for the honor that you have bestowed upon me, and I assure you that I will do all in my power to carry out your wishes.

Thank you. [Applause]

PRESIDENT BRUECKNER: Doctor Garrett, it is a pleasure to receive you as the Second Vice President of this Association.

DR. H. U. GARRETT: Thank you, Doctor Brueckner.

Ladies and gentlemen, it is certainly a great pleasure to accept this appointment as a step up the ladder, and I want to thank each and every one of you. I assure you I shall do all in my power to help this Association and its officers in the future.

Thank you. [Applause]

PRESIDENT BRUECKNER: Francis, it is a great pleasure to greet you as the Third Vice President of this organization. You come from the extreme Northeast, but you are familiar with the problems all across the country because of your breadth of vision and understanding.

MR. F. G. BUZZELL: I want to voice my appreciation to this Assembly for this honor, and I hope I will be able to help advance the aims of this Association for the benefit of the livestock industry.

Thank you. [Applause]

PRESIDENT BRUECKNER: Again I would like to express my appreciation, and I feel that as far as I am concerned the job is done. I shall now turn the meeting over to our new President, Dr. George Good.

[Dr. George Good assumed the Presidency.]

PRESIDENT GOOD: Thank you again, Doctor Brueckner. If there is no further business to come before this meeting, we will stand adjourned.

[The meeting adjourned sine die at 12:45 p.m.]
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 two kinds of members—Official and Individual. The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, The Territories, 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. Any person engaged in livestock sanitary work for federal, provincial, state, territory, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.

ARTICLE IV—MEETINGS
The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS
The officers of this Association shall be: President, First Vice-President, Second Vice-President, Third 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 and Territories, the
Chief of the United States Bureau of Animal Industry, the Veterinary Director of Canada, the executive regulatory officer of Cuba and Mexico, The Territories, Puerto Rico, the Virgin Islands, Los Angeles County, California and the elective 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 First Vice-President 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.

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 Fraction of the Program Committees; 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. First Vice-President: The first vice-president 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. 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 and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the first vice-president. He shall be an ex-officio member of the Executive Committee.

4. Third Vice-President: The third vice-president shall assume the duties of
81 the president in the event of the absence, disability or resignation of the presi-
dent, first vice-president and second vice-president. He shall assume the chair-
manship of the Executive Committee in the event of the absence, disability or
resignation of the first and second vice-presidents. He shall be an ex-officio
member of the Executive Committee.
86 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 ap-
proved by the Association.
94 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 Com-
mittee. 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.

102 Article VIII—Amendments

103 The constitution of this Association may be amended by a two-thirds vote of
104 the members of the Association present and voting at an annual meeting, pro-
vided that the specific amendment to be acted upon shall have been presented
in writing at a previous annual meeting and further provided that the amend-
ment has received the approval of the Executive Committee.

108 By-Laws

109 Article I—Order of Business

110 Registration.
111 Call to Order.
112 Report of Secretary-Treasurer.
113 President's Address.
114 Reading of Papers.
115 Committee Reports.
116 Discussion.
117 Unfinished Business.
118 New Business.
119 Nomination and Election of Officers.
120 Adjournment.
121 A suspension of the By-laws made 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 Live-stock 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.
Five 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 official memberships shall be fifty dollars ($50.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
SIXTY-FIRST
ANNUAL MEETING
CHASE PARK PLAZA HOTEL
St. Louis, Missouri
November 13-14-15, 1957