Committee on Anaplasmosis—K. J. Peterson, Salem, Oregon

4:25 Discussion

4:30 Problems of State Veterinarian in Controlling Poultry Diseases—Ralph L. West, D.V.M., St. Paul, Minnesota

4:50 Report of the Committee on Transmissible Diseases of Poultry—M. S. Hofstead, Ames, Iowa

5:00 Executive Committee Meeting
Committee on Legislation
W. L. BENDIX, Richmond, Virginia, Chairman
R. A. HENDERHOTT, Trenton, New Jersey
J. A. MCCALLAM, Washington, D. C.
H. A. MILI, Harrisburg, Pennsylvania

Committee on Tuberculosis
G. B. RAE, Pierre, South Dakota, Chairman
T. D. BRANDELBURG, Bismarck, North Dakota
J. CANTY, Montpelier, Vermont
W. N. CLIFF, Boston, Massachusetts
C. E. KORD, Nashville, Tennessee
J. A. RANKEY, Washington, D. C.
J. W. SOUTHERN, Helena, Montana
J. E. STUART, Sacramento, California
E. TIBBETTS, San Juan, Puerto Rico

Committee on Vascular Diseases
R. A. BANKOWSKI, Davis, California, Chairman
F. CAMARGO, N. Obregon, Mexico D. F.
J. W. MANN, Atlanta, Georgia
D. G. MOTT, Minneapolis, Minnesota
F. J. MULLER, Falls Church, Virginia
J. TRAUN, Greenport, Long Island, New York
K. F. WELLS, Ottawa, Ontario

Committee on Parasitic Diseases
F. R. KUOTZ, Columbus, Ohio, Chairman
D. C. ROUGHTON, Wilmington, Delaware
E. L. CURRY, Kansas City, Missouri
J. HOUVRIGAN, Washington, D. C.
H. E. KEMPER, Albuquerque, New Mexico
T. D. SCHWARTZ, Washington, D. C.
L. E. SWANSON, Galveston, Florida

Committee on Nominations
R. L. WERF, St. Paul, Minnesota, Chairman
C. E. KORD, Nashville, Tennessee
K. J. PETTERSON, Salem, Oregon
R. W. SMITH, Concord, New Hampshire
K. WELLS, Ottawa, Ontario

Committee on Transmissible Diseases of Poultry
M. S. ROPES, Ames, Iowa, Chairman
A. CHRISTIE, Kingston, New Hampshire
J. F. DELAPLAIN, College Station, Texas
E. M. DICKENSON, Corvallis, Oregon
J. F. WITTEY, Oro, Maine
H. E. GOLDSMITH, Columbus, Ohio
P. J. LAYTON, Haam, New York
J. W. MANN, Atlanta, Georgia
C. J. STEWART, Los Angeles, California
D. S. POMEROY, St. Paul, Minnesota

Committee on Public Health
J. E. STUART, Sacramento, California
F. J. F. BURTON, Seattle, Washington
J. W. CARVER, Madison, Wisconsin
J. W. SMITH, Atlanta, Georgia
J. J. STEELE Atlanta Georgia

Committee on Public Relations
J. A. RUSSELL, Jackson, Mississippi
C. L. CAMPBELL, St. Louis, Missouri
W. J. JENKINS, Pearl River, New York
R. L. KUOTZ, Columbus, Ohio
C. D. VAN HOUWELING, Oaxton, Virginia

REPORT OF THE EXECUTIVE COMMITTEE

Method of conducting business—Motion, second, and a three-quarters vote of the members present shall be required for action.

THURSDAY, NOVEMBER 14

9:00 Report of the Advisory Committee to Agricultural Research Service—U.S.D.A.—W. L. BENDIX, Richmond, Virginia
9:15 Further Studies on Enzootic Vesicular Stomatitis—R. P. Hanson and D. A. Schenkel, Madison, Wisconsin

10:00 Discussion
10:25 Report of Representatives to Annual Meeting of Commissioners, Secretaries and Directors of Agriculture—R. W. Smith, Concord, New Hampshire; R. A. Hendersott, Trenton, New Jersey; W. L. BENDIX, Richmond, Virginia

10:55 Discussion
11:00 Veterinary Medical Education—Mark Allam, V.M.D., Philadelphia, Pennsylvania
11:30 The Evaluation of Anthrax Spore Vaccine (Non-encapsulated) In Sheep—P. G. White, Ph.D., Madison, Wisconsin
Proceedings
SIXTY-FIRST
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

CHASE-PARK PLAZA
St. Louis, Missouri
November 13-14-15, 1957
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OFFICERS 1957 - 1958

J. F. MILLIGAN  
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*1st Vice-President*

R. A. HENDERSHOTT  
*Secretary-Treasurer*

JAS. R. HAY  
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L. J. Poelma, College Park, Maryland
T. O. Roby, Silver Spring, Maryland
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D. Ibsen, Little Rock, Arkansas
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K. J. Peterson, Salem, Oregon          K. F. Wells, Ottawa, Ontario

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G. H. Good, Cheyenne, Wyoming            H. J. Rollins, Raleigh, North Carolina
                                      O. Sussman, Princeton, New Jersey

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M. S. Shahan, Greenport, Long Island, New York
B. Shambaugh, Jr., Denver, Colorado
J. Traum, Berkeley, California
K. F. Wells, Ottawa, Ontario

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NATIONAL ASSOCIATION OF STATES DEPARTMENT OF AGRICULTURE

DELEGATES
A. A. Erdmann
H. G. Geyer
R. L. West
R. A. Hendershott

ALTERNATES
L. Davisson
A. K. Merriman
J. Green
## Record of Previous Meetings

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<thead>
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<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tbody>
<tr>
<td>1. Sept. 27–28, 1897†</td>
<td>Forth 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. 22–24, 1902</td>
<td>Wichita, Kansas</td>
<td>*Mr. W. H. Dunn, Tennesseee</td>
<td>*Mr. Wm. P. Smith, Monticello, Illinois</td>
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<tr>
<td>No.</td>
<td>Date Range</td>
<td>City, State</td>
<td>Name, City, State</td>
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<tr>
<td>34</td>
<td>Dec. 3-4-5, 1930</td>
<td>Chicago, Ill.</td>
<td>*Dr. A. E. Wight, Washington, D.C.</td>
</tr>
<tr>
<td>36</td>
<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
</tr>
<tr>
<td>37</td>
<td>Dec. 6-7-8, 1933</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
</tr>
<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, Wis.</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>
<tr>
<td>61</td>
<td>Nov. 13-14-15, 1957</td>
<td>Chicago, Ill.</td>
<td>Dr. G. H. Good, Cheyenne, Wyoming</td>
</tr>
</tbody>
</table>

* Deceased.
† This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
‡ Reprinted in 54th Annual Report.
WELCOME TO ST. LOUIS

L. A. Rosner
State Veterinarian of Missouri

DR. L. A. Rosner [Jefferson City, Missouri]: President Good and Members of the United States Livestock Sanitary Association:

Just a few minutes ago I learned that Mr. Williamson, our Commissioner of Agriculture, could not be here because of illness, and of course I knew earlier that the Governor could not be here.

On behalf of Governor James T. Blair, Jr., and John S. Williamson, Commissioner of Agriculture, I want to extend their regrets at their inability to be with you today, and welcome you to St. Louis and Missouri and to wish you success in your meeting.

Here in Missouri the livestock industry is our largest single industry. Seventy-five percent of our agricultural income is derived from livestock and poultry. With the reactivation and acceleration of our disease control programs during the past four years, I know that our livestock industry has a better appreciation of the importance of your organization and the contributions you have made over the years toward our knowledge of livestock diseases and toward our methods and procedures for controlling and eradicating those diseases. Certainly as State Veterinarian I know very well the importance of your work and the contributions you have made.

So, again, on behalf of the Governor and the Commissioner, I want to extend to you our welcome and our very best wishes for a successful meeting. We hope you will leave with much new knowledge of livestock disease. We trust that your stay here will be pleasant.

Thank you. [Applause.]

President Good: Thank you, Doctor Rosner.
RESPONSE TO WELCOME

DR. F. B. WHEELER

Baton Rouge, Louisiana

DR. F. B. WHEELER: Mr. President, Doctor Rosner, Officers and Members of the United States Livestock Sanitary Association, Ladies and Gentlemen:

Doctor Rosner has made it pretty easy for me. I can spend more time responding.

I have been attending these conventions for the last 10 years, but this is the first time I have ever sat through this opening service. I had no idea of the importance and seriousness of this part of the program; in fact, as I see it, we start off with the least important address first (that's the President's) and we build up to the address of welcome by Doctor Rosner, and everything is sort of keynoted to the response I am about to make. [Laughter.]

Before I go any further, I have noticed that Doctor Bendix is a little fidgety. If you want to go out, Bill, go ahead. You won't hurt my feelings at all. [Laughter.]

I want the Association to know that I fully recognize that the success of this convention depends a great deal on this response, and I have spent a great deal of time preparing this spontaneous response that I am going to give this morning. In preparing it I wanted it to be brief, but at the same time I wanted it to be expressive but concise, and at the same time I wanted it to convey sincere sincerity. That's the best kind. At the same time I didn't want to provoke any uncontrollable emotions.

With this prelude, Doctor Rosner, I would like to say, on behalf of the United States Livestock Sanitary Association, that we sincerely appreciate your address of welcome, and we want to assure you that we have every intention of taking advantage of every opportunity to enjoy our visit here in St. Louis.

That's it. You have to admit it may not have been as good a speech as I said it would be, but it certainly was brief, and it didn't provoke any uncontrollable emotions. [Laughter.]

No kidding, Gentlemen. When Ralph asked that I make this response, what I just delivered to you was what I thought he had in mind. I had planned on a little more physical response when Doctor Rosner got through, but by the time he finished I was so stagestruck that I couldn't even whistle. I didn't bring my whistling teeth with me. I might have borrowed Doctor Rollins', but he was using them. At least I can still talk with a Southern drawl. It would have been terrible if I had borrowed Rob Smith's teeth. [Laughter.] I had no idea that they made rock-'n-roll teeth in North Carolina. [Laughter.]

My only excuse for not knowing how to make a response to an address of welcome is that we are on the other end of this business and are sort of isolated. We are continually being bombarded with these awful Texas
Jokes from the West and the Voice of America programs from the East, and it is only occasionally that we get an off-color joke that trickles down to us from Arkansas.

Speaking of Arkansas, I notice Doctor Joe Campbell is not here. Evidently he was not successfully smuggled through the Federal lines. [Laughter.] I understand he made it but they caught his stomach. Being more than a little attached to it, he gave up his freedom and went back.

Seriously, I know Doctor Campbell has a round with his heart and is a little under the weather, but I understand he is well on his way to recovery and will be back with us next year. We certainly miss him. I think Doctor Hendershott should send him a “get well” card and a fifth of Old Granddad. He can mail the card and I’ll be glad to take the fifth. [Laughter.]

I didn’t realize until last Friday that I was going to have 10 minutes to give this response, so I thought I would do some research and find out how the Yankee responders have responded in the past. It soon became obvious that what they had to say was irrelevant, immaterial and had little to do with the address of welcome of the man preceding him.

I also did some research on Missouri, but I couldn’t go along with the information they were trying to deal out. The almanac I was reading said that Missouri was noted for mules, St. Louis Women and Budweiser Beer. That I now go along with; but then they said that there had been a tremendous drop in the number of mules during the last 10 years, and in the same paragraph it said there had been an increase in beer production. As everybody knows, that is an impossibility. I just couldn’t swallow that. [Laughter.]

Seriously, Doctor Rosner, I can see why you are proud of Missouri, because you seem to have achieved what so many states in the South are striving for, and that is a good, healthy balance between agriculture and industry. As you said, you have made tremendous strides in livestock disease control in the last four or five years, and I know you know that we are serious about that, because Doctor Pass, the Federal veterinarian, has made two trips up to Jefferson City in the last year to pick up a few pointers and to profit by your mistakes. I must say that we did learn a lot on our trip, but we didn’t see any obvious major mistakes. If they were made they were pretty well covered up. I am interested in how you do cover them up, because I swear that Doctor Pass has pulled some lulus in Louisiana. [Laughter.]

Doctor Pass and I appreciate the tremendous amount of time that you spent with us on our trip to Jefferson City, and the time you spent so intelligently answering the stupid questions we asked.

I am going to learn a lot on this trip, too; in fact, I have already picked up a dirty story from Jean Smith. [Laughter.]

Again, Doctor Rosner, we appreciate your warm words of welcome, and I want to assure you that the “Show Me” State is going to show us a good time, and that we will enjoy ourselves here.

Thank you very much. [Applause.]
PRESIDENT'S ADDRESS

G. H. Good

Cheyenne, Wyoming

Members of the United States Livestock Sanitary Association, distinguished guests, ladies and gentlemen, speaking both for myself and the officers of the Association, it gives me extreme pleasure, to welcome you to our sixty-first annual meeting.

It has been a long time since our group met in St. Louis—1904, to be exact, and since we have been moving about it is entirely fitting that we should again meet here. To most of us, St. Louis suggests the early days of the fur trade and we overlook that it was founded nearly 200 years ago, in 1764. We remember that it was a military supply depot for the early days of the west, the starting point for many of the exploratory and military missions, and fail to connect it with the first public kindergarten in connection with public schools. Here on the west bank of the Mississippi, 20 miles below the confluence with the Missouri, 200 miles above the entry of the Ohio, and 1,270 miles above the Gulf of Mexico, we are going to find St. Louis does things to this water that really improves it.

INTERSTATE REGULATIONS FOR THE MOVEMENT OF LIVESTOCK

The veterinarians and livestock men look to the United States Livestock Sanitary Association for guidance in the interpretation of the interstate regulations on shipment of livestock. The handbook put out as a compilation of existing regulations is eagerly looked forward to and frequently consulted. Unfortunately, no sooner is it in print than its contents are subject to change.

Every regulatory official must frequently encounter this question: “Why can’t other states have the same regulations we do? It would certainly facilitate shipment of livestock if you regulatory people got together and decided upon a uniform set of regulations.”

There is no simple answer to this question. Uniform regulations throughout the country would certainly be ideal. The United States Livestock Sanitary Association has striven towards this goal, but, in trying to reach it, sometimes complicating factors are inadvertently added when our livestock regulatory officials return home. Such uniform regulations, while desirable, are impracticable because methods of stock raising differ so widely in different parts of the country. So long as we overlook this major factor, the only way we can arrive at uniform interstate regulations is to make them so complex they will satisfy the most demanding state. Other states, not seeing the need for such rigid regulations, will begin to relax their requirements until within a short time a whole new variable set of regulations again exists.
However, if we realize that within a geographical area conditions are pretty much the same irrespective of state boundaries, there is a hope that uniform regulations might be achieved on an area basis. Certainly 10 neighboring states are more apt to agree than 48, and one is a little more inclined to make concessions to a neighbor for the cause of uniformity than to a state half a continent away.

To this end, I recommend the committee on laws and regulations discuss the feasibility of establishing uniform regulations by areas. While subdivision may have to be carried further, men would be appointed representing the western states to include Texas, Kansas, Oklahoma, Nebraska and the Dakotas; the north central states; the southern tier of states and the eastern states.

Should this committee fail to be able to work out any acceptable procedure in the near future, or even as an adjunct to a successful arrangement, further consideration should be given to the binding of these interstate regulations in loose leaf form. Then if changes must be made and each state will retain the page size of the booklet, keeping this booklet up to date will be simplified.

**Tuberculosis**

None of us question the desirability of eliminating bovine tuberculosis. For long years now we have been depending upon the intradermal tuberculin test, accrediting counties or areas when the amount of infection was below certain levels. Our thinking must be changed here, without any reflection on the accuracy of the tuberculin test. If we are going to eliminate tuberculosis, either a more intensive application of this test, or other methods will have to be employed. We are going to have to search out the infected animal. This was really the original purpose of the area tuberculin test, but the sampling techniques frequently employed since then quite often have tended to hold the disease within bounds and produce an optimistic frame of mind as to the prevalence of bovine tuberculosis. This has progressed to the point where some states which have intensified their own campaigns now refuse to accept cattle from modified accredited tuberculosis free areas without the added guarantee of an immediate negative tuberculin test of animals being shipped. This attitude certainly is not to be endorsed but reflects the thinking of those who see the complacency of other areas in applying the tools we have in combating the disease.

Barring acute losses from disease, practically all of our cattle eventually are slaughtered. The purebred dairy bull, the worn out beef bull, and the aged dairy cow, as well as those animals produced primarily for slaughter will eventually be butchered. Considering the large numbers of animals killed at young ages, probably the average age of a complete turnover of the cattle population is at five years. Within a period of five years, then, the entire cattle population could be subjected to a thorough post-mortem examination on the killing floor. This plan of attack is subject to two factors. First, will the animals slaughtered be examined to determine if tuberculosis is present?
Statistics show that in 1937, 71 percent of the cattle which were slaughtered were inspected at establishments where Federal inspection was maintained. This figure dropped to 63 percent in 1947 and during 1957, 73 percent of the cattle were adequately inspected to determine if tuberculosis was present. In addition to the cattle slaughtered at establishments maintaining Federal inspection a large number were slaughtered at either municipal or State establishments with adequate facilities for post-mortem inspection.

The production of wholesome meat and meat products—apart from any consideration of tuberculosis—dictates that such inspection services be made available wherever public slaughter is maintained. Without going into further detail, even the smaller establishments can avail themselves of properly trained veterinary services on a part-time basis for this. Secondly, if mere recognition of tuberculosis lesions is the end, such procedures become entirely passive. The animals must be so identified that the source of infection can be returned to and measures taken to stamp out the focus. This identification program can be used for a similar approach to other disease problems and should be expanded to other classes of meat.

While the tuberculin test should still be employed in dairy cattle, purebred animals, reactor herds and wherever else feasible such a program of killing floor inspection would be more useful than testing the minimum number of negative range cattle to limp through for accreditation of a county.

During 1957 there were 8,976,409 head of cattle tuberculin tested, of which 13,974 were reactors. The approximate cost in locating one infected animal by testing alone was $490.00. No national figures are available of the approximate cost of tracing back to the herd of origin tuberculous animals found on the killing floor. However, New York State reports their approximate cost was $32.25 per animal.

BRUCELLOSIS

Considerable progress towards the eradication of brucellosis has been made in the past three years, although, understandably, much remains to be accomplished in the ensuing three years to reach the national goal of eradication by 1960.

From July 1, 1953 to July 1, 1954, 9,002,109 cattle were tested for brucellosis and 2.6 percent were found infected. For the comparable period of 1956-1957, 15,913,396 cattle were tested with 1.7 percent infection found. In 1954, modified certified brucellosis free states included North Carolina, New Hampshire and Maine. In addition to these three, Connecticut, Delaware, Minnesota, Washington, Wisconsin and Vermont are now in this category for a total of nine, plus 475 counties in 25 other states and Puerto Rico. However, in spite of this marked progress over three years, this will have to be increased by five fold in the next three years. Undoubtedly, many states will require the full three years to reach their individual goals but steady progress from year to year will be heartening. In the same way, continued effort toward lowering the percentage of infected animals disclosed should be expected, but it should be remembered that more work may have
PRESIDENT'S ADDRESS

to go into decreasing the number on the right of the decimal than has gone into lowering the digit on the left as more and more tests have to be conducted to find a reactor.

The recently activated Federal requirements for the interstate movement of cattle with respect to brucellosis have assisted materially in emphasizing that the day for living with brucellosis is past. These requirements preventing the interstate movement of brucellosis infected cattle have been effective in the screening of a good many cattle in themselves but even more important they have indicated to many states that this eradication program is an active one that will not be confined to a few dairy states but is far reaching in its results.

With brucellosis control we have had the advantage of several means of approach and test modifications, plus a highly effective vaccine. Eventual eradication is not going to be a simple matter and to this end we should explore all possible means of combating the disease. As we evaluate such measures we may find that, while some are less effective than others, they fit admirably into certain situations and are thus worth retaining.

I hope it is not premature at this point to encourage a little forward thinking as to continuation of the program after 1960. Certainly, even with what we term eradication, there will be no time in the near future when we can stop thinking of brucellosis control. The final years of this program are taking considerable money and it is essential to remember that more will be needed after 1960. Are we going to continue to get or even to accept assistance from a benevolent Federal Government? In our messages to the livestockmen we have stressed the economic benefits of control. Are the State and Federal Governments to continue supplying free vaccine and free administration? Can this program eventually be financed by the livestockmen alone when we utilize dry and cull cow testing at the markets and on the killing floor without his explicit request for this service? Fortunately, less attention need be directed to these questions over the next three years than in achieving our 1960 goal, but they should not be entirely overlooked.

HOG CHOLERA

Hog cholera was reportedly first seen in Ohio in 1833 and by the late 1850's had become a major problem. Tremendous losses were taken from this disease until the development of the simultaneous method of vaccination utilizing serum and virus. This would have seemed to have been the answer to the problem, for certainly the immunity produced by this procedure has been well established. However, losses from this major swine disease continued, and it became apparent that in some instances such vaccination procedures were carrying on the disease. Modified and inactivated virus vaccines have been with us long enough to indicate that they can adequately replace the older serum and virus procedure. An added step in the control of hog cholera has been the banning of raw garbage feeding as the result of the vesicular exanthema outbreaks a few years ago. The stage has thus been set for a serious attempt at the elimination of hog cholera. Looking
at the problem broadly, each source of infection could be derived from: (1) An existing outbreak of hog cholera. (2) The feeding of garbage containing virus. (3) The use of unattenuated virus. If we can eliminate one of these factors we should be able to eliminate the others. This does not mean we will have to leave our hogs without a biological barrier against hog cholera because these newer vaccines have been amply proven to be satisfactory. By removing two of these factors we will cut down upon the third factor, the active disease itself.

Fourteen states have discontinued the use of virulent hog cholera virus. The results of their action as well as the test pilot procedures in Florida will be followed with interest.

**ANAPLASMOSIS**

It is customary for the president to review those livestock diseases of regulatory concern or of an unusual nature. It would appear that anaplasmosis might well fall into this category for it is of incidence in 39 of the 48 States at the present time. This is a disease of chronic nature where the infected animal tends to remain a source of infection for its associates. Reports would indicate that its incidence is increasing and our modern methods of transportation combined with the insidious nature of the carrier animal itself would suggest this to be entirely possible. With the development of the complement fixation test, control measures are feasible but continued research into therapeutic measures against anaplasmosis is justified. With any disease, appreciation of its distribution is important so that one of the primary steps is to determine the extent of its spread. Fortunately the complement fixation test is especially applicable to such surveys.

**DIAGNOSTIC AND RESEARCH FACILITIES**

We have been free of foreign exotic diseases this past year but we must become increasingly aware that modern means of transportation can quickly introduce many of these undesirable diseases. We have the facilities of the Plum Island Animal Disease Laboratory for work on our recognized exotic diseases, but where can consultation and laboratory assistance be secured for other presumed foreign diseases of highly infectious nature. Strengthening of our local diagnostic centers should certainly be encouraged and the current attempt to catalogue and list their facilities is commendable, but it is hoped that the new Animal Disease Laboratory at Ames, Iowa, will be in a position to complement their efforts when the need should arise. The development of plans and specifications for this laboratory are in the final stages and bids for the construction of the facilities will be submitted early in 1958. It is anticipated that actual construction will start by next spring—the goal for completion is early 1960. Sometimes research projects seem far afield from regulatory edicts, but all of the latter to be successful have been derived from facts secured from research. The impetus given to research in diseases of livestock is an encouraging sign that the need has been recognized, but to reap the full benefits it should be further encouraged.
PRESIDENT'S ADDRESS

APPRECIATION

Election to the presidency of the United States Livestock Sanitary Association has been a great honor for me, but the capable assistance of Dr. Ralph Hendershott, the Association's secretary, is sincerely acknowledged. Doctor Hendershott is the man who keeps our organization going from year to year in that all important interval between meetings. My thanks and appreciation also are extended to the committee members, our speakers, and the other officers for their help in making this sixty-first session the success it promises to be.
PRESENTATION OF KEY TO RETIRING PRESIDENT GOOD

R. A. HENDERSHOTT

Trenton, New Jersey

SECRETARY HENDERSHOTT: As usual, this is the happy time of the meeting for me, for two reasons. One is that we are approaching the end of a very successful meeting as far as program is concerned; second, it is a delight for me to have the opportunity and the responsibility of presenting to our retiring Presidents a memento of the service they have rendered the livestock industry and this Association during their term as President of the Association.

I think this year our President has had his share of glory. He has worked hard here at the front desk, and his bouquets now grace the alcove on either side of the stage.

Those of you who have been here since Wednesday know that for the preceding two days we have had two beautiful baskets of flowers here, one on either side of the rostrum. I have been in this Association for quite some time, and this is the first time in my recollection that any President has been so honored by not only his colleagues in the hall but by the farm people whom he serves back home.

On top of that I must tell you that he received a two-page telegram, the like of which any one of us could be proud of. I suggested to him that he have it plasticized and hung in his office. Certainly this is an exceptional case. I didn’t know that our President was so well thought of outside our own group. Of course we have known about him here, and we hold him in high regard; but having public recognition come from the folks back home, it really was something. Personally, I would be proud to receive such an emolument as that.

Doctor Good, in just a very minor way we wish to try to leave with you a token of our appreciation of the efforts you have given on behalf of the Association during this year of your Presidency. You have done an admirable job. You have followed in the footsteps of your predecessors, and in some measures I think you have excelled them.

It is a pleasure for me to present to you this very slight token of our appreciation of the services you have rendered the livestock industry and the United States Livestock Sanitary Association, and we wish for you many, many more years of service with us. [Applause.]

[President Good accepted the key.]

PRESIDENT GOOD: Thank you very much, Doctor Hendershott, and gentlemen. This key will remind me of your trust and confidence in selecting me to head this great organization. It will also repeatedly awaken me to my appreciation of the cooperation and assistance everyone has so kindly given me during my term of office.
REPORT OF THE SECRETARY-TREASURER

R. A. HENDERSHOTT
Trenton, New Jersey

SECRETARY HENDERSHOTT: Mr. President, Members of the United States Livestock Sanitary Association and Friends:

It just occurred to me that it has been some fifty-three years since our Association held its last meeting in this city. In 1904, St. Louis was the host city for the eighth annual meeting of the Interstate Association of Live Stock Sanitary Boards. At that time Dr. J. C. Norton was President of the Association and Dr. D. F. Luckey State Veterinarian of the State of Missouri was responsible for bringing the meeting here, and took a prominent part in the program.

Since then we have traveled a great deal around this nation, and it took us fifty-three years to get back to St. Louis. I hope perhaps some time we will be able to go back to Chicago for a while, provided we don't run into a deal like the one we had there last year. Frankly, I think we ought to stay within a 500-mile radius of the City of Chicago, since that is the hub of the livestock industry. However, some of our members think differently. I don't particularly object to traveling, but I do think we can serve the industry better if we stay within a reasonable radius of the members' homes and farms.

At this time I want to express my sincere thanks to the chairmen of the various committees who worked so well with us during the past year, and whose responsibility it is (along with the officers of this Association) to develop the programs that we have at our annual meetings. In addition to that, many of our committees work very hard both at the convention and between conventions on matters of important policy. That is as it should be.

One area in which we have failed to really do a job, in my opinion, is in getting the contributors to our program to go along with us in preparing two copies of their paper for the Secretary's office and having them in his hands by the middle of September at the latest. The reason we like to have the papers in advance is that it provides an opportunity to our Committee on Public Relations to review the papers, to abstract them, and to have mimeographed handouts prepared for the press, and thus to get the ultimate out of public relations with the press, radio and television.

I think we are missing a big bet by not cooperating more closely in this respect. For this particular meeting and at this time we had three papers presented to us between the 1st and 10th of September—and there are something like sixty papers on the program of this meeting.

We utilize the services of the host state veterinarian (Dr. L. A. Rosner in this instance) to head our Public Relations Committee because he is on the
ground floor and can make contact with the radio and television stations in
the area.

This year we are greatly handicapped due to the fact that we have not
received the papers in advance, and there is little we can do in the way of
getting out publicity on the meeting and satisfying the press in that con-
nection. The only thing we can do is to continue to encourage speakers
to get their papers in early, and to invite the press to attend some of our
sessions and hear the papers as they are given.

We have also called upon publicity people in the area, and we are very
appreciative of the fact that they are contributing their time to assist us in
this very important area of public relations. That also goes for the other
members of the Committee who are working very hard in this respect.

This year we had a little difficulty—it seems that we have had a lot of
difficulty this year, for one reason or another—because the organization
that has been printing our programs for the last 10 years retired the printer
who had always handled the printing of our programs. About half of the
programs had been sent out when I discovered that they had printed the
Wednesday morning program, following it with the Thursday morning and
Thursday afternoon program, and on the reverse side had printed the Wed-
nesday afternoon and Friday morning programs.

This created quite a bit of confusion. I immediately called it to the
printer’s attention. Fortunately we retrieved the envelopes that had been
addressed to the state regulatory officials and to the Commissioners, Sec-
retaries and Directors of Agriculture, and saw to it that the programs that
went to those people were corrected.

We had a reprinting of the entire program in blue, as you can see. I
didn’t think it was necessary to readdress envelopes to about 600 members,
many of whom would not attend the meeting, so we let the first batch go
out. I found out later that some members were confused. One man wired
me from California and said he had both programs but he couldn’t figure
out which one to follow. He happens to be on the program and he wanted
to know when he should arrive here. One other speaker didn’t know when
he was supposed to be here, and he had to change his reservation.

I apologize sincerely to the membership for this error. We should have
cought it. I should have read the program, but I had confidence in the
printer because he had been printing our program for many years and
nothing had ever gone wrong before. The galley proof was all right, but
when the man began setting up the type he made the mistake. We will
watch this hereafter. Having had this one experience, we will see to it that
it does not happen again.

Annually for the last ten years or so I have spoken to you people about
memberships. Through deaths, resignations and retirements we drop about
150 members annually. Through the efforts of you members we pick up
just about enough to keep our membership on a par. For example, in 1955
we had 1,081 members, which seems to have been the peak. In 1956 we
dropped to 1,018 members. Currently we have dues from 1,064 members
for this current year. I would be happy to be able to say we have 10,640 members. I suppose as long as I am Secretary of this Association I shall never be satisfied with our membership figures.

Last year we asked you to approve Junior memberships, and this matter will come up later in the meeting, for final decision. I sincerely hope we will have your cooperation and acquiescence in providing for Junior memberships.

As I said, I would like to see around 10,000 members in this Association instead of 1,000. It would be a simple matter to increase our memberships if each of us would devote just a little effort to it. If each one of us sold one person we could double our membership. You know how it works out on the $64,000 quiz program. It will work out that way in our Association if we will just get on the ball and do the job.

Again, my thanks to the officers of the Association, from whom we have had fine cooperation this year, and to the membership-at-large, and particularly to the chairmen of our various and sundry committees for their efforts during the past year.

I am happy to report we are in good financial condition, as you can see. If there are any questions about this report, I will be happy to answer them. Also, I would suggest that an Auditing Committee of at least three members be appointed to audit the books of the Treasurer. Thank you.

PRESIDENT GOOD: Thank you, Doctor Hendershott.

Before we go on with our meeting, I would like to call your attention to the beautiful baskets of flowers on each side of the podium. These are sent with the compliments of the Wyoming Livestock Sanitary Board. I was surprised and very honored when I learned that these flowers were here, and I am deeply grateful not only to the Livestock Board but also to the Wyoming Stock Growers Association.
REPORT OF THE AUDITING COMMITTEE

R. W. SMITH, Concord, New Hampshire
H. G. GEYER, Columbus, Ohio
A. P. SCHNEIDER, Boise, Idaho

DOCTOR SMITH: Mr. President, Ladies and Gentlemen: Your Auditing Committee has attended to its duties. We find the Association in good financial condition, and the books in order in every way.

There isn't much more that our Committee can say. We are pleased to inform you that the finances have improved each year. Thank you.
MEMORIAL SERVICE
M. N. RIEMENSCHNEIDER
Oklahoma City, Oklahoma

President Good, members of the Association, ladies and distinguished guests: To the best of my information, the following members have passed away since last we met:

FRED R. BEAUDETTE

Dr. Fred R. Beaudette (KSC '19), 59, Chairman of the Department of Animal Pathology at Rutgers University, New Brunswick, New Jersey, passed away on January 16, 1957. Doctor Beaudette was a graduate of Kansas State College. After graduating he served on the faculty at Kansas State College until 1923, at which time he joined the Rutgers’ staff. The Doctor was outstanding in poultry pathology work. He received world-wide acclaim in the perfection of vaccines against Newcastle disease and laryngotracheitis. In 1944, he won the Borden Award for his methods of controlling the latter disease. In 1951, he received the Doctor of Science degree from Rutgers University. Doctor Beaudette was a member of a number of professional and scientific societies. He was a fluent writer—having published many papers.

JOHN W. CHILDS

Dr. John W. Childs (COL '28), 59, Denver, Colorado, died December 2, 1956. Doctor Childs graduated from Colorado A. & M. College where he received his degree in Veterinary Medicine in 1928. He practiced veterinary medicine in California for eighteen years. He served in the Armed Forces in World War II and returned to Denver in 1951 where he worked with the United States Public Health Service. He was named Assistant State Veterinarian of Colorado in 1954 and was appointed State Veterinarian in 1955, in which capacity he served until his untimely passing.

CHESTER F. CLARK

Dr. Chester F. Clark (MSU '29), 57, passed away on July 28, 1957. Shortly before his death the doctor became Dean Emeritus of the College of Veterinary Medicine, Michigan State University. He attended Massachusetts State Agricultural College from 1919 to 1921. He then enrolled at the former United States College of Veterinary Surgeons in Washington before entering Michigan State where he received his D.V.M. Degree in 1929. Following graduation he became a member of the pathology staff at the college and had been a fulltime faculty member ever since except for a three-year period, 1946-1949, when he served as State Veterinarian of Michigan. Doctor Clark was made head of the Department of Surgery and Medicine in 1949 and was named
dean in 1951. He directed the planning and reorganization in connection with the veterinary college's move into its new and greatly enlarged facilities, then under construction. His research activities were in the field of dairy cattle disease, especially, reproductive disorders. He was author of a number of articles on these conditions. Doctor Clark was a member of a number of professional organizations.

CHARLES HENRY CLARK

Dr. Charles Henry Clark (ONT '90), 89, of Lansing, Michigan, passed away on August 23, 1957. Doctor Clark was a graduate of the Ontario Veterinary College in 1890. He was appointed Assistant State Veterinarian of Michigan in 1915. In 1930 he was named Chief Veterinarian and retired in 1946.

LEE SEGHETTI

Dr. Lee Seghetti (WSC '43), 47, Fort Collins, Colo., died at his home on November 20, 1956. He had been a member of the veterinary faculty at Colorado A. & M. College since 1954. Doctor Seghetti received his B.S. Degree at Washington State College in 1935, his D.V.M. in 1943, and an M.S. degree at Oregon State College in 1948. He was assistant veterinary bacteriologist there in 1938-39, assistant bacteriologist and pathologist with the United States Fish and Wildlife Service in 1939-1941, and a graduate assistant in pathology in the veterinary college at Washington State in 1941-1943. He then served as pathologist in the veterinary research laboratory at Montana State College until 1954. In the latter year, he joined the veterinary faculty at Colorado A. & M. College as parasitologist. Doctor Seghetti held membership in a number of scientific and honorary societies, was a collaborator in the animal disease and parasite research branch, Agricultural Research Service, U.S.D.A., since 1949, and was author and co-author of numerous papers and bulletins on parasitic diseases of range sheep and cattle, of fur-bearing and game animals, and clostridial diseases of sheep.

R. Q. SMITH

Mr. R. Q. Smith, 58, of Columbus, Ohio, passed away during the year. He was Secretary of the Independent Marketing Association of Ohio and was instrumental in establishing principles of sanitation and disease control in livestock markets in his native state.

DR. JOHN P. DELAPLANE

Dr. John P. Delaplane (OSU '29), 50, passed away in Bryan, Texas, on September 22, 1957. Doctor Delaplane received his degree in Veterinary Medicine at Ohio State University in 1929, where he also received his M.S. Degree in 1931. From the time of graduation until 1942 he did research work at the Rhode Island Extension Service for four successive years. Thence he was Pathologist at the Texas Experimental Station from which he returned
to Rhode Island as Animal Pathologist Chief and again returned to Texas in 1950. At the time of his death he was Chief of veterinary microbiology in the College of Veterinary Medicine, Texas A. & M. Doctor Delaplane was widely known for his research in poultry diseases. He was a prolific writer. He received world-wide recognition for his contribution in the control of poultry diseases. Doctor Delaplane was a very active member of the Association having served on many committees and having made many contributions.

DR. H. U. GARRETT

Dr. H. U. Garrett (KCVC '17), 64, Des Moines, Iowa. Doctor Garrett passed away on April 2, 1957. He graduated from Kansas City Veterinary College in 1917. Immediately after graduation Doctor Garrett established a practice in St. Charles, Iowa, where he practiced for the next 34 years. After retiring from general practice in 1947 he became State Veterinarian of Iowa. He continued to serve in this capacity until his untimely death. Doctor Garrett was highly respected in the field of regulatory medicine and was very active in the practitioner’s group in his state. He served on many committees of this organization and was second vice-president at the time of his passing.

DR. F. P. WILCOX

Dr. F. P. Wilcox (GWU '16), 64, Los Angeles, Calif., passed away April 13, 1957. Doctor Wilcox received his degree in Veterinary Medicine from the George Washington University in 1916. The same year he accepted an appointment as Veterinary Inspector with the Bureau of Animal Industry, U.S.D.A. and was assigned to Chicago on ante-mortem and post-mortem inspection. In 1917 he accepted an appointment as Deputy State Veterinarian for the State of California. During his services with the State, he was assigned to livestock disease control and also to milk and dairy sanitation. He served as field veterinarian and senior field veterinarian until September, 1927, at which time he accepted a position as Veterinary Inspector of Los Angeles County Health Department. In 1953, he was appointed Los Angeles County Livestock Inspector, in which capacity he served until his death. He was active in the California State Veterinary Medical Association and many national organizations.

Will all present please rise and remain standing for a short period of silent prayer for the peaceful repose of the souls of our departed colleagues.

Thank you, ladies and gentlemen, for your respectful participation.

Your speaker feels very humble in attempting to properly memorialize these great men. It would take someone with a great deal more talent than I to properly convey our deep feelings and emotions on this occasion. They are gone, but they have left behind a great inspiration. They have contributed immeasurably to veterinary science, especially the principles of disease control. Their road was not easy: they knew disappointments, sorrow, and worry—knew the pain of being misunderstood. But they had the courage,
the stamina, and the foresight to pursue truths, to overcome the obstacles and the pitfalls in their paths. Let us not falter in progressing toward these goals so ably set forth by them. In their passing we have lost personal friends and colleagues and many helpful services performed by them, but we will not have lost the inspirational aid for which they were so well known and appreciated. The world will be a better place in which to live for their having passed this way. Graciously we memorialize them today and let us resolve to emulate the goodness and grandeur their lives so well displayed.
Dr. R. W. Smith [Concord, New Hampshire]: Mr. President, Members and Guests of the Association:

The meeting of Commissioners, Secretaries and Directors of Agriculture, held in the Hotel Wentworth-by-the-Sea, Newcastle, New Hampshire, just four miles outside of Portsmouth, on September 30 through October 3, was attended by your three delegates—the speaker, Doctor Hendershott and Doctor Bendix. In addition, the Director of Animal Industry of the State of Maine, Commissioner Buzzell, and Dr. T. J. Grennan, State Veterinarian of Rhode Island, were present. Doctor James R. Hay was there but in his capacity as a Commissioner. Last year, when he was here, he was the State Veterinarian of Ohio.

The meeting was well attended. New Hampshire, as you might expect me to say, went all out to entertain the delegates. The members worked very hard on Monday and Monday night, but on Tuesday we took them in buses up into the White Mountains, back to the University of New Hampshire, where they were given a chicken barbecue at seven o'clock in the evening and then they returned to the hotel and worked diligently all day Wednesday and Thursday forenoon, and then left for their homes.

There was one feature of the meeting which I doubt is a regular affair. It intrigued me. I presume it was due to the energy and foresight of my boss, Commissioner Fitts. All meals to everyone were sponsored by various national organizations. Believe me, such a program does more for the appetite of a spindling man than you could ever imagine. We even had fried chicken for breakfast one morning, and all the dairy products we could eat.

Concerning the business of the meeting, we sat in on the Committee on Animal Diseases. However, before I touch on that I want to report that the net result of their meeting was that they passed some 28 resolutions.

One subject in particular interested your delegates, and I believe it will be of interest to this Association. To bring you up-to-date on it I will quote from a letter sent to the regulatory officials by our Secretary, Doctor Hendershott, which states in part:

“At a meeting of the North Central group of State Commissioners in July, 1957, the following resolution was passed and recommended to the entire Association:
"WHEREAS, it has been the often expressed concern of most Commissioners, Secretaries and Directors of Agriculture over the years with the multitude of associations and conferences created and attended by subordinate state employees, and

WHEREAS, these associations, made up of employees of state departments of agriculture, have developed into policy and action groups who resolute, recommend and act in various official manners—often without sanction or knowledge of the respective department heads, and

WHEREAS, the regional and National Associations of State Departments of Agriculture have become more active and effective in the past few years, showing leadership and capacity to handle such matters, and

WHEREAS, we need to present a unified and consolidated front on all matters of concern affecting departments of agriculture; therefore, be it

RESOLVED: That this North Central Association, here in convention at Starved Rock, Illinois, seek support of the National Association this fall, and the other three regional associations at their earliest opportunity, in setting up an over-all Association of State Departments of Agriculture, with all subordinate officials being organized into affiliated associations which meet, recommend and report only to the parent group made up of the Department heads (presently the National Association of Commissioners, Secretaries and Directors of Agriculture)."

This resolution was discussed. They set up a panel discussion. The delegates made it very clear that this organization was not made up in its entirety of subordinates to the Departments of Agriculture, and in fact only some 32 states were operated under a Commissioner of Agriculture, that is, the disease end of the state's activities, and there were some 14 or 15 states in which the Director of Animal Disease was under the direct orders and supervision of a board, separate and apart in its entirety from the Department of Agriculture.

Also, that our membership was now about 1,100, and naturally is was made up of breeders, ranchers, dairymen—in fact, our Constitution states anyone who is affiliated or interested in animal disease eradication, milk sanitation and other related factors, and that we would act as a clearing-house for scientific research that was gathered from time to time, and that we also act as a clearing-house to disseminate through our committees and through the holding of hearings, and so on, this information so that it will get into the hands of those whose duty it is to see that the diseases of the animals in this country are kept to a minimum, and in many cases completely eradicated.

I am happy to report to you that, with only one or two exceptions, they were very quick to recognize the fact and to point out to us that this organization was not one that they had discussed, and they had no idea of including it in their category of discussion.
I might tell you that this arose out of one branch of their departments that foolishly went to Washington and interceded for legislation entirely opposite to that which the Commissioners had agreed upon. You know how you would have felt if you had been a Commissioner.

This started back in San Francisco in 1955, so they have been discussing it for at least a couple of years.

So much for that report. Now may I read the resolution that they passed before they adjourned, relative to disease eradication. Your delegates were invited and urged to participate, and did so in their panels and discussions, as freely as we do here.

[Doctor Smith read the resolutions, paper marked No. 22.]
COMMISSIONERS, SECRETARIES AND DIRECTORS OF AGRICULTURE
RESOLUTIONS
(By R. W. Smith)
No. IX
BRUCELLOSIS ERADICATION PROGRAM

WHEREAS, the eradication of brucellosis program in many states is making rapid strides; and

WHEREAS, brucellosis still remains a major disease control problem in several states; and

WHEREAS, because of increased interest on the part of the United States Department of Agriculture has resulted in the States stepping up activity and appropriations in order to meet the States' share of the financial responsibility, cooperating with the United States Department of Agriculture’s activities and appropriations; and

WHEREAS, the present Federal authorization for brucellosis funds is in the amount of 20 million dollars annually, said funds being borrowed at the present time from Commodity Credit Corporation; and

WHEREAS, said authority expires on July 1, 1958, after which date no authority will exist to continue to borrow funds of the said Commodity Credit Corporation; and

WHEREAS, an appropriation for the accelerated brucellosis testing program will have to be authorized by the Congress of the United States in a new bill; and

WHEREAS, such bills, namely, H.B. 8152 and Senate Bill 2408, are now pending in Congress, and it is imperative that these bills be passed in order to continue the accelerated brucellosis testing program;

THEREFORE BE IT RESOLVED by the National Association of Commissioners, Secretaries and Directors of Agriculture, assembled at Portsmouth, New Hampshire, September 30 to October 3, 1957, that this Association urge the Congress of the United States to pass H.B. 8152 and S.B. 2408;

BE IT FURTHER RESOLVED that this Association requests the United States Department of Agriculture to assist in every way possible toward the passage of said bills and the continuation of the brucellosis program;

BE IT FURTHER RESOLVED that a copy of this resolution be sent to the Chairmen of the Appropriations and the Agriculture and Forestry Committees of the Senate and the Chairmen of the Agriculture and the Appropriations Committees of the House of Representatives at Washington, D.C., and further that a copy be sent to the Secretary of Agriculture of the United States Department of Agriculture;

BE IT FURTHER RESOLVED that each member of this Association urge his Senators and Representatives to support the said bills.
COMMISSIONERS RESOLUTIONS

No. X

HOG CHOLERA SUPPRESSION

WHEREAS, for over one hundred 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 diseases; and

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

WHEREAS, the continued cooking of garbage lends itself strongly to the control of hog cholera; 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 National Association of Commissioners, Secretaries and Directors of Agriculture, assembled at Portsmouth, New Hampshire, September 30 through October 3, 1957, urges the Federal Government to work with the States in developing a plan for controlling and eradicating hog cholera with the continued cooking of garbage; and

BE IT FURTHER RESOLVED that if it develops there is a need for additional information in regard to this disease before proceeding with the eradication program, research and trial projects be conducted to produce the information needed.

No. XI

LEPTOSPIROSIS CONTROL

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

WHEREAS, there are no approved uniform diagnosis, treatment and control procedures on the local, state and national level;

THEREFORE BE IT RESOLVED by the National Association of Commissioners, Secretaries and Directors of Agriculture, assembled in Portsmouth, New Hampshire, September 30 through October 3, 1957, 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.

No. XII

SWINE BRUCELLOSIS CONTROL PROGRAM

WHEREAS, brucellosis in swine is a serious and costly disease confronting swine producers; and

WHEREAS, the fine progress being made in the control in the 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 National Association of Commissioners, Secretaries and Directors of Agriculture in convention assembled at Portsmouth, New Hampshire, September 30 through October 3, 1957, urges its Executive Committee and the United States Department of Agriculture to develop and recommend to the States a cooperatively supported and financed program for the control and eradication of swine brucellosis.

DOCTOR SMITH [continuing]: Mr. President, this is our report as delegates. We suggested to the representatives that it would be very beneficial if their organization would send two or three delegates to attend our meeting, and they agreed 100 percent. I presume Doctor Hay was one who was appointed, but due to a death in his family he is not here. If there are other representatives here, I have not met them as yet. However, they did receive the invitation from us to send representatives to our meeting. Thank you. [Applause.]
REPORT OF THE ADVISORY COMMITTEE TO THE AGRICULTURAL RESEARCH SERVICE


The Advisory Committee to the Agricultural Research Service has held a series of four meetings during the past year. Two of these meetings were held in Washington, D. C., one in Cleveland, Ohio, and one here in St. Louis. The Committee wishes to express its appreciation to the officials of the Agricultural Research Service for their cooperation and help throughout the year. All phases of regulatory and research programs have been discussed with the appropriate officials of the Agricultural Research Service.

New and anticipated programs have been studied and discussed in detail so that a thorough understanding has been reached on all major points concerning these new undertakings. The officials of the Agricultural Research Service have furnished the Committee with all information and material that has been requested by the Committee and have kept the Committee informed of all the work and changes in their organization.

There has been one major change in the organizational set up of the Agricultural Research Service during the past year. This change raised the officials in the Agricultural Research Service in official titles so that they now hold positions in the Department of Agriculture in keeping with the positions that are held by other officials in the Department.

One new major program has been started during the year and one new section has been established in the Agricultural Research Service. The new major program that has been started during the year is that of the eradication of the Screw Worm Fly in cooperation with the State of Florida. This Committee has been kept advised as to the progress of this program. The new section to be set up in the Agricultural Research Service is one for the control of poultry diseases. This is a forward step in the opinion of the Committee and one that will serve a much needed purpose.

The Committee has been kept informed of all pending legislation before the Congress and of the Department of Agriculture's official position concerning such legislation.

The tuberculosis eradication program has been discussed at length and we are advised that there has been a decrease in those counties that are delinquent in their accreditation.

The Committee feels that this subject should be investigated further by the Committee on Tuberculosis and that necessary steps be taken to have these counties brought up to date. The Committee has studied and discussed with the Agricultural Research Service Officials problems of no visible lesions cases in tubercular reactor animals. It is the feeling of the Committee that
this problem now endangers the entire tuberculosis eradication program and that some change in the nomenclature of these cases should be made. It is suggested that the words "no gross lesions" be used instead of the present designated "no visible lesions." The Committee feels that the tracing of animals with tuberculous lesions to farms of origin has been of great assistance in the control of the disease and recommends that this program be strengthened.

The scrapie program has been discussed and it is the feeling of the Committee that this program needs considerable study and revising.

The Committee has carried out the recommendations of the Association pertaining to hog cholera and has requested that a swine disease control program in cooperation with the States be established in the Agricultural Research Service.

A thorough discussion of the inspection and quarantine service has revealed that this program is under-staffed and under-financed to adequately do the job that is expected of it and have recommended that this program be strengthened in order to carry out the purposes for which it was intended.

The research program has been discussed by the Committee with officials in charge of this program. The Committee feels that at the present time, this program is adequately staffed to conduct that part of the program that is possible with the limited physical facilities now available. The Committee is cognizant of the fact that there will be a serious shortage of personnel when the new Research Laboratory is opened at Ames, Iowa. For this reason, the Committee recommends that funds be made available now to hire and train new personnel at State-owned Institutions so that this personnel can be moved to Ames when that laboratory is finished. This step would allow us to receive the maximum benefits at the earliest possible time from the new laboratory.

We find that there is possibly a greater need for expanded financial aid to the meat inspection program than to any other program in the Agricultural Research Service. There has been continued demand by the consuming public that this service be increased. The rise in cost of operation has caused a decrease in personnel to render such service. There has been no increase in the budget to carry out these services. If these services are to continue at the high level that is expected by the consuming public, there must be an increase in the budget for this purpose.

The budget for the fiscal year beginning July 1, 1959, has been discussed with officials of the Agricultural Research Service and the Committee's opinion concerning that budget was presented to the budget review committee. The following recommendations were presented to that committee:

The Advisory Committee of the United States Livestock Sanitary Association is deeply concerned with the present state of affairs in the Meat Inspection Division. This is with special regard to the lack of personnel and the increasing number of plants which are coming under Federal inspection.
It is noted with concern that as new plants request meat inspection and that as meat inspection is granted this spreads out the present personnel very thinly and thereby slows down the speed of production at some plants. Also it is noted that as employees grades or normal salary increases occur, this restricts the hiring of new personnel on a limited budget. In one year 71 new plants in 35 towns requested and received Federal inspection. Meat inspection is now in effect in 471 towns or cities. It is our understanding that about 100 plants are now requesting Federal inspection and that they meet the requirements, but Federal inspection is slow in coming, due to the lack of personnel and funds.

Any cut in funds or refusal of increase in this Division actually reflects itself in the limiting of the Division in its normal mode of operation.

It is also noted that the chain stores and military requests for inspected meats are gaining rapidly and consumer knowledge has demanded inspected meats.

The Committee recommends that the amount appropriated for the eradication of tuberculosis be continued in the 1959 budget.

We also recommend that the amount of $4,150,000 be appropriated in the budget for the eradication of brucellosis, and we further strongly recommend that a request be made that the transfer of $20 million per year for the 1959 and 1960 fiscal years from the Commodity Credit Corporation be continued. The Committee feels very strongly that it would be disastrous to the brucellosis program if the availability of funds for the extensive brucellosis eradication campaign now in progress is not continued. The Committee also recommends that a section or branch be established in the Animal Disease Eradication Division for the control and eradication of diseases of swine, and that a minimum of $1 million be appropriated to establish and activate such a section or branch. Your Committee further recommends that an appropriation of at least $750,000 be appropriated for the diagnosis, control, and eradication of miscellaneous diseases. It seems vital that this amount be available in case of emergencies of numerous kinds which may appear at any time.

We further recommend the transfer of $240,000 from Commodity Credit Corporation funds for the continued work in the eradication of vesicular exanthema until such time as a section or branch for the eradication of swine diseases has been established. The Committee is most gratified that the Department has recognized the great lack of laboratory facilities available for necessary research in livestock and poultry diseases. We hope that the construction, staffing and operation of the laboratory at Ames will go forward with speed and dispatch.

The list of diseases and parasitic conditions that plague the livestock and poultry industry is growing almost daily. The current budget request for research is realistic in that it makes the fullest use of existing facilities but it is anything but realistic as measured against actual need.
The Committee urges that budget consideration be given promptly to the need for training additional personnel to adequately staff the Ames Laboratory when ready. It is vital that this training be started at once and continued as long as needed.

The facilities of the nation's land grant colleges and universities are available to the Department if adequate funds are provided for this function. The Committee feels that an additional sum of not less than $300,000 annually must be provided for this service.

Another division the Committee is keenly concerned with is the Animal Inspection and Quarantine Division.

It is noted in the serum and virus production that there is a complete changeover in the last 10 years from killed to live products, also that in the last 10 years production has climbed 1,000 percent.

In 1945 total doses produced 288 million.
In 1955 total doses produced 2,207 million.

As these products are mostly new products and relatively untried, this creates many problems in determining whether a product is safe and can be effectively used for its intended purpose.

One prime example is the shift in the production and use of hog cholera serum and virus to the use of attenuated hog cholera virus.

Your Advisory Committee received a slight criticism from the budget review committee in that their recommendations were too near in line with the request that had been presented to them by the Agricultural Research Service. Your Committee answered this criticism by explaining that the problems at hand were so large and the expected budget allotments were so small that only the major needs had been requested and that as these major needs were overcome, there would be additional requests and recommendations made that might not conform with the thinking of the Agricultural Research Service.

The Committee recommends that in the future the Advisory Committee to the Agricultural Research Service be composed of five members. These members shall be the First Vice-President of the United States Livestock Sanitary Association and one member selected from each of the four District Geographical Regions of the United States. We recommend further that the First Vice-President of the United States Livestock Sanitary Association serve as Chairman of this Committee.
I

REPORT OF COMMITTEE ON LAWS AND REGULATIONS

J. W. Green, Chairman, Indianapolis, Indiana; H. G. Geyer, Columbus, Ohio; T. C. Green, Charleston, West Virginia; A. K. Merriman, Springfield, Illinois; M. N. Riemenschneider, Oklahoma City, Oklahoma; R. L. West, St. Paul, Minnesota.

Your Committee on Laws and Regulations reporting in Chicago called attention to USDA regulation Title 9 which was to become effective January 1, 1957. The purpose of this regulation is to prevent the introduction of brucellosis through the interstate movement of cattle.

Doctor West, chairman of the Committee, called for the cooperation from the States in its enforcement of existing State regulations. It was the expressed desire of the Committee that Title 9 should be reviewed at this meeting with special emphasis on progress of enforcement and needed changes if any. Your Committee has reviewed Title 9 through the medium of a questionnaire which was sent to all chief livestock sanitary officials and discussions held in connection with this meeting.

II

ENFORCEMENT

Most states reported that enforcement is proceeding at an acceptable rate. Many states report confusion on the part of the livestock people, but this is to be expected with the inauguration of any new regulation be it Federal or State in origin. In most states, enforcement is being conducted cooperatively and in practically all instances the information and educational phase has preceded the prosecution phase. I am sure we all agree that this is the best approach to gaining compliance. A few officials feel that enforcement should be stepped up.

III

PREEMPTION OF STATE REGULATIONS

Since the preemption of State regulations was the main objection raised at Chicago, it was believed to be of interest to determine how many states had sought legal advice on this point and the results of such inquiry. Only nine states reported they had investigated this point, but the results were generally the same. It is the opinion generally that no state can interfere with cattle moving interstate which are in compliance with Title 9. All are of the opinion though that once cattle have come to rest inside a state's boundaries, state laws would prevail.

It has been pointed out however that in the case of the interstate TB regulation State regulations which have been more restrictive have been enforceable.
IV

Since it is conceivable that if Title 9 will do the job (that is—prevent the spread of brucellosis through the movement of cattle) it might some day be the standard regulation by which cattle are moved intra- and interstate, an opinion was sought as to whether Title 9 could obtain the desired effect if unsupplemented by State regulations. Only a few officials, most of them in western states, felt Title 9 alone could do the job. This same question was framed in a different way—"Could Title 9 do the job after the nation is certified and assuming an effective test trace back from slaughter program for beef cattle is perfected and the ring test used to the fullest?"

The answer was still no.

RECOMMENDATION

After studying the suggestions submitted by the various livestock sanitary officials your Committee recommends the following:

1. Enforcement efforts be continued and intensified where necessary.
2. That consideration be given requiring all cattle moving interstate to be accompanied by a health certificate issued by an accredited veterinarian except animal moved to an approved stock yards or slaughtering establishment.
3. Care should be used so as to not leave the impression that only Federal regulation need be complied with.
4. That the A.R.S. further explore the possibility of tying in the State regulations with Title 9.
REPORT OF THE COMMITTEE ON LEGISLATION


The first session of the 85th Congress, which convened in January, 1957, considered a great many bills of either general or specific interest to this Association but enacted only a very few of them into law. There were no less than 10 separate bills introduced dealing with the use of humane methods in the slaughtering of livestock and poultry intended for interstate and foreign commerce. These bills followed the same general pattern and while their general intent is sound and commendable, it is the opinion of this Committee that the subject requires a good deal more careful study before effective legislation can be introduced and passed.

After much compromise and debate the Congress passed a Poultry Products Inspection Act which became public law 85-172 when it was signed by the President on August 28, 1957 (71 Stat. 441). The Secretary of Agriculture immediately placed the enforcement of this Act in the hands of the Poultry Division of the Agricultural Marketing Service. Proposed regulations authorized by this Act have been drawn and public meetings already have been scheduled or held throughout the nation dealing with these proposed regulations. The Poultry Products Inspection Act is possibly as good a law as could be gotten at this time. It does not represent the thinking or the recommendation of this Association, and it falls short of what the professional workers in this feel are necessary minimum requirements, but at the same time it is a tremendous step forward. How the Act will be administered by the Agricultural Marketing Service is yet to be determined. This Committee recommends that careful and continuing study be given both the Act itself and the manner in which it is administered so that factual data may be assembled which can be used to correct any weaknesses that may develop in the law itself or the efforts used in its administration. In other words this Committee feels we should give the Act itself, and the Agricultural Marketing Service every chance to provide the nation with an acceptable Poultry Products Inspection Service, and failing this, we should again take the matter up with the Congress.

In March of this year a bill was introduced by Mr. Grant of Alabama (H.R.-5933) to control the preparation, distribution, importation and exportation of Virulent Hog-Cholera Virus. This bill did not prohibit the production of this material, but it prohibited its importation or use within the United States except under special permit from the Secretary of Agriculture. The Secretary was directed to issue such permits only if he determined that hog cholera occurred so regularly or to such an extent in
REPORT OF COMMITTEE

the areas under consideration that the use of this product would not materially increase the hazard. This bill was called for hearing before the House Committee on Agriculture and supported by all the professional opinion present. No action was taken, principally due to opposition raised by an individual hog feeder from the State of Delaware. The Committee recommends that this Association go on record as favoring legislation of this type aimed at the ultimate disappearance of virulent hog-cholera virus from the field of immunization.

S-2192 and companion bills designed to clarify the language and intent of certain laws granting the Secretary of Agriculture authority to control and eradicate contagious, infectious diseases were introduced but not acted upon. This legislation contemplates increasing the Secretary's authority to seize and destroy livestock and livestock products in interstate commerce when there is any threat to the livestock in the United States present. Section 2 of this legislation extends the Secretary's authority to seize and destroy such stock on any premises in the United States whether or not there is any interstate movement involved. Under this legislation this may be done whenever the Secretary declares an extraordinary emergency and he notifies the state of his intentions. This section has provoked considerable debate. Sound reasons can be advanced for placing the ultimate authority to act in the hands of the Secretary, and at the same time equally sound reasons can be put forward that the police powers possessed by the individual state are sufficient for any conceivable emergency. The Committee seeks guidance and advice from the Association in this matter.

Legislation designed to extend the financing at the current rate of the Accelerated Brucellosis Program beyond the present expiration date of June 30, 1958, was introduced and as yet has not been acted upon. From its beginning the financing of this program has been by the transfer of funds from the Commodity Credit Corporation, which is primarily intended for commodity stabilization. The present position of the Department of Agriculture is that continued use of the Commodity Credit Funds beyond June 30, 1958, for brucellosis eradication would not be proper. The Department favors the continuation of the Accelerated Brucellosis Program, but feels it should be financed by increased appropriations to its regular Disease Control Funds for this purpose. This would involve nearly a five-fold increase in the regular Brucellosis Appropriation which in view of the current feeling of both the Administration and of the Congress regarding increased appropriations, seems highly unlikely of accomplishment. This Committee strongly recommends that this Association wholeheartedly indorse the Accelerated Brucellosis Program again, and that it urge a continuation of the program until its completion, and that it support every effort designed to obtain sufficient Federal financing to accomplish this purpose.
REPORT OF THE COMMITTEE ON PUBLIC RELATIONS

L. A. ROSNER, Chairman, Jefferson City, Missouri; C. L. CAMPBELL, St. Louis, Missouri; W. JENKINS, Pearl River, New York; R. L. KNUDSON, Columbus, Ohio; C. D. VAN HOUWELING, Oakton, Virginia.

SECRETARY HENDERSHOTT: Next on the program normally would be the report of the Committee on Public Relations. Since the man who served so admirably as Chairman of the Committee during this meeting was called back to Jefferson City, Missouri, he has asked me to make a report for him.

I might say that we had considerable difficulty at this particular meeting in trying to organize a program on public relations because of the fact that the contributors to our program failed to turn in their papers by September 1 as requested. I know that in some instances work is being carried on on these subjects, and it is impossible to meet a deadline that far in advance of our meeting.

However, if we are going to do a job of public relations in this Association—if we are to do the ultimate we would be able to do at our meeting—it is imperative that our contributors, when they are requested to prepare a presentation for our meeting, are informed to have two copies of their presentation typed and in my hands not later than September 15. It takes time to go over the papers and abstract from them those things that are considered newsworthy.

From experience as a veterinarian I know that we in this business of veterinary medicine seldom know what is news. We have to have an agricultural editor go over the papers and abstract from them the material he knows will appeal to the public, whether it be the farm public or the urban public. This year we weren’t able to do that job, and more effort was expended at this meeting than at prior meetings due to the fact that we were not supplied with copies of papers in advance of the meeting.

In spite of that I think we owe a very sincere vote of thanks to Doctors Rosner and Van Houweling and others who assisted them in getting out the material for the press, and in making contacts for radio tapes and television appearances.

Therefore, I would ask you men who are going to serve on our committees as chairmen that when you are contacting those who are going to present papers on our program, will you please impress upon them the necessity of their sending in two copies of their paper well in advance of the meeting.

You will recall that at the meeting in Chicago and the meeting prior to that, Dr. R. L. Knudson of Ohio did a splendid job with his co-workers in developing mimeographed abstracts to be handed out to the press. That left more time to select persons to make tape recordings, and so on. In any event, we are indebted to him, and I hope we can impress upon him that we need his services when we meet in Miami Beach next year.
VETERINARY MEDICAL EDUCATION

MARK W. ALLAM, V.M.D.

Philadelphia, Pennsylvania

Veterinary medicine is that branch of medical science which serves agriculture. Despite the fact that veterinary education in the United States has been predominately associated with agriculture, veterinary medicine should not be regarded as an agricultural science. Indeed, if the profession is to serve agriculture well its educational program must develop as a part of the biological and medical sciences.

That unrest exists in medical education today, including veterinary medical education, is recognized by most educators. Several leading medical schools have recently embarked upon educational programs which differ markedly from the traditional approach that we have known during the past 25 years. These experiments in medical education have a common goal: To meet the future demands of the rapidly changing dimensions of medical science. Veterinary medicine faces the same challenge and we must begin to examine critically all aspects of our educational fabric. There are many problems which must be considered in developing a future program for veterinary medical education and not all of them can be mentioned here. A few of the more important problems are:

1. The need for developing more correlative teaching in our curriculum.
2. The need to provide additional opportunities for students to develop their particular interests.
3. The need to provide additional opportunities for graduate education.

At present the educational program in veterinary schools is built almost entirely around the individual departments. Each department teaches its own particular course or group of courses without much regard for what is being taught in other departments. Our curriculums are generally arranged to provide for a progression through basic science courses to subjects in clinical science. This provides the opportunity for some continuity of subject matter but unless there is an organized approach to integration of material, there is a tendency on the part of the student to view each course as a separate entity. As a result, the student is often left with a maze of facts but with no clear idea of how they may be correlated. There is also a problem of repetition of subject matter and in our already crowded curriculum this can result in a serious waste of time. For these reasons there is much interest today in the development of correlative teaching programs. One distinct advantage of the correlative approach is the fact that subjects can be covered in greater depth. For example, in our present program the anatomy and physiology of the kidney and the diseases and pathological changes affecting this organ are considered in widely separated places in the cur-
VETERINARY MEDICAL EDUCATION

Curriculum. In a correlative program it would be possible to consider all of these areas in a closely integrated fashion so that the student would have an immediate appreciation of how each is related to another. One further example of meaningful integration would be the condensation of small and large animal medicine into a single course. Medicine is medicine, whatever the species, and with this approach the really fundamental basis of medical practice could be studied in depth. It is far more important for the student to understand the underlying causes of disease than to memorize groups of non-specific signs and symptoms. In summary, a correlative program of teaching offers certain advantages which seem worthy of exploration by those interested in veterinary medical education. Part of the success of any venture in correlative teaching will depend upon the creation of an administrative structure conducive to this type of program. That is, it will be necessary to provide a structure which is flexible enough to allow for a free interchange of teaching effort between the various disciplines. At present it is often difficult to achieve any degree of correlation because of over-departmentalization.

The veterinary medical curriculum of the future must provide more opportunities for the capable to develop his particular interests. Until a few years ago the singular aim of schools was in developing veterinary practitioners. Little or no thought was given to developing the veterinary medical scientists. Today there is an increasing need for veterinarians qualified in special fields of research, public health, teaching, regulatory medicine and in allied fields of medical and biological sciences. Veterinary medical education has an obligation to provide the broadest possible background for students who wish to enter these particular fields.

There are other reasons for broadening the student's educational background. The veterinary physician in farm practice is finding fewer farms with larger total numbers of animals per farm, thus creating a greater responsibility for maintaining herd health. This calls for an increased emphasis in our curriculum on principles of preventative medicine.

Our present curriculum is so crowded and rigid that it is difficult to provide the opportunities for students to pursue special interests or to introduce new material which would broaden our educational perspective. In providing the future means of accomplishing these objectives, I do not advocate increasing the time required to complete a course in veterinary medical studies. Rather, there is a need for critically examining our present curriculum with the aim of determining areas which can be reduced in scope, so that new material can be introduced. This is a difficult problem but we cannot allow our curriculum to become static if we are to meet the challenges of the future.

In recent years veterinarians have shown an increasing interest in the development of certain clinical specialties. It is doubtful whether the veterinary medical profession will ever approach the medical profession in its degree of specialization. However, where there is a desire to obtain special training the opportunities for doing so should be made available. Many faculties
now have individuals who are recognized for their contributions in clinical specialties, and some veterinary schools are offering short courses in clinical subjects for the graduate veterinarian. At the School of Veterinary Medicine, University of Pennsylvania, courses in such subjects as Dermatology, General Surgery and Orthopedic Surgery have been offered for several years. These are short, intensive courses designed to offer the latest information in these particular clinical fields. In addition, some schools now offer a specific curriculum for veterinarians who wish to do graduate work in a clinical speciality.

These are only a few of the problems facing veterinary medical education today. It is not the intent of this paper to provide answers but rather to define some of these problems. Those interested in education have the obligation to make a concerted effort in developing a curriculum which will meet the rapidly changing scope of veterinary medical science.
REPORT OF THE COMMITTEE ON REGULATORY EDUCATION

MARK ALLAM, Chairman, Philadelphia, Pennsylvania; W. W. ARMISTEAD, College Station, Texas; W. R. KRILL, Columbus, Ohio; A. K. KUTTLER, Salt Lake City, Utah; R. S. SUGG, Auburn, Alabama.

Each year it seemingly becomes more difficult to present the Committee report on regulatory education without special reference being made to a previous report of this Committee. It would seem appropriate to quote a paragraph from the 1956 Committee report.

"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."

Also in last year’s report the Committee pointed out the increased participation in applied regulatory education as evidenced in the communications from the various schools and colleges of veterinary medicine.

The faculties of the schools and colleges of veterinary medicine should accept additional responsibilities in regulatory education beyond the instruction of the veterinary medical student. For example, the sponsoring of symposia on animal diseases as a refresher course for those members of our profession associated with Federal, State or Municipal Government in the health and regulatory services. At this time the latest concept of animal disease as it affects the host can be discussed as well as many important public health problems as they relate to the regulatory veterinarian. Toward this end you will be interested in a "Poultry Disease Short Course" which was held at Iowa State College October 21-November 1. There are 22 men working in the poultry disease field in all sections of the United States who are ARS employees. It is the understanding of the Committee on Regulatory Education that this is the first attempt to have a specialized short course covering this length of time for any ARS people at a college of veterinary medicine. Several schools have sponsored symposia of one or two days and it is hoped additional meetings will be planned for the future.
REPORT OF COMMITTEE ON RESOLUTIONS

Dr. A. K. KUTTLER, Chairman, Salt Lake City, Utah; Dr. R. W. CARTER, Columbia, South Carolina; Dr. J. S. CAMPBELL, Little Rock, Arkansas; Dr. T. J. GRENNAN, Jr., Providence, Rhode Island; Dr. R. H. SINGER, Frankfort, Kentucky; Dr. W. F. FISHER, Reno, Nevada.

Mr. President, distinguished guests and members of the Association: Your Committee on Resolutions has been in contact with each other since their appointment and have written to all State and Federal regulatory officials requesting them to submit suggestions for consideration by your committee on resolutions in the interest of improving our service to the livestock industry and consumers of food of animal origin.

We have received many suggestions and referred them to the various committees of the Association who in our opinion should handle them.

The following were adopted by your resolutions committee:


1. That the United States Livestock Sanitary Association convey its thanks and appreciation to the officials of the City of St. Louis for the warm reception, hospitality and courtesies extended to us by the officials and others in this great city. We are especially indebted to the Ralston Purina Company for the courtesies extended to us. The complimentary trip to their farm and the fine dinner served to us was enjoyable and educational.

2. We extend our sincere appreciation and gratitude to our personable and congenial Secretary Dr. R. A. Hendershott for his efficient and untiring efforts in behalf of the Association.

3. We convey our sincere appreciation and gratitude to Mrs. Charlotte Emmons for her excellent service in recording the proceedings of our annual meetings which keep us current on matters pertaining to diseases of animals.

4. We express gratitude and appreciation to each speaker who participated in the program and the officers of the Association for arranging such an interesting and inspirational program, and to members of the various committees who in many instances labored far beyond the call of duty in crystalizing the thinking of the Association and preparing reports which will be of lasting value to us as we read them and to those of our membership who could not be with us, as well as the entire livestock industry which will profit through the information accumulated for them in these reports.
RESOLUTIONS

RESOLUTION REFERRING TO PERMITS PRIOR TO SHIPMENT OF LIVESTOCK INTERSTATE

WHEREAS, many State Livestock Sanitary Officials now require livestock owners to obtain, in addition to health certificates, a permit from the State Livestock Sanitary Officials of destination prior to shipment of their livestock, and

WHEREAS, the obtaining of such permits are granted solely upon the information supplied by those at the point of origin and the purpose is to give advance notice to the sanitary officials of the arrival of such livestock, and

WHEREAS, prompt submission to the State Livestock Sanitary Officials of the State of destination of a copy of the Health Certificate via Air Mail at the same time copy of the Health Certificate is delivered to the shipper would serve the purpose of the prior permit and at the same time reduce delay and expense to the shipper while waiting for the permit,

NOW THEREFORE, BE IT RESOLVED, that except in the case of dairy cattle, all State Livestock Sanitary Officials change their regulations or laws as soon as it can be done so as to accept an Air Mail copy or telegram pertaining to livestock being shipped or moved into their State in lieu of the permit.

RESOLUTION PERTAINING TO BRUCELLOSIS ERADICATION

WHEREAS, the livestock industry of this country petitioned the Federal Government to assist them in the control and eradication of brucellosis which was at that time (1934) the number one communicable disease of livestock in this country, and

WHEREAS, the only source of brucellosis in man is from domestic animals, and

WHEREAS, all of the States have joined with the Federal Government in the control and eradication of this serious disease of animals and man at great cost to them, with the results that nine whole States and many counties in other States have achieved the status of modified certified brucellosis free areas, and

WHEREAS, failure to pursue the campaign to a successful conclusion would have the effect of placing in jeopardy the great financial investment thus far made, the herds and areas thus far freed from the disease, and, more important than all, discourage the ideal of eradicating instead of living with communicable diseases of animals:

NOW THEREFORE, BE IT RESOLVED, that the Secretary of the Association be instructed to write the Secretary of Agriculture, United States Department of Agriculture and the Secretary, Director and Commissioner of Agriculture in each of the States furnishing them a copy of this resolution and request them to present this matter to the members of Congress who will, when properly informed, not want to break faith with livestock owners and others who have labored for many years in an effort to eradicate this serious disease of livestock and protect the health of their families on farms and ranches who are in a most vulnerable position with regard to brucellosis, now that victory is so near.
STATISTICAL DESIGN FOR DISEASE INCIDENCE*

D. S. Robson, Ph.D. and James A. Baker, D.V.M., Ph.D.

The need for accurate information on the regional and national incidence of the various livestock diseases becomes apparent when we examine the economic aspects of a livestock vaccination program. Vaccination of all young livestock in a given agricultural region is economically efficient only if the total cost of vaccination is less than the total dollar loss which the region would incur from the disease without vaccination. The efficiency of a vaccination program may therefore be determined from the ratio:

\[
\frac{\text{total loss}}{\text{total cost}} = \frac{\text{number of diseased animals} \times \text{average loss per diseased animal}}{\text{number of animals} \times \text{cost per vaccination}}
\]

Incidence of the disease, as well as the experimentally determined average loss per diseased animal, is thus essential to the objective evaluation of livestock vaccination.

A national survey in which veterinarians are enlisted to collect livestock serum samples in the course of their routine practice provides an immediate and practical means of acquiring this necessary information. A statistical design for such a survey follows, and the expected accuracy of the resulting disease incidence estimates is indicated.

**Proposal for a Disease Incidence Survey.** In over-all design, the proposed survey may be described as a stratified quota sample, with each of the 48 States constituting a stratum. It is intended that serum samples shall be collected by enlisting the aid of a number of veterinarians from each State, and that each such cooperator shall be requested to collect a specified quota of samples. Serum samples will be mailed to the serology laboratory of the nearest of seven selected veterinary colleges. Each of these seven laboratories will be provided with a technician and the equipment necessary to process and store each sample. The laboratories do not represent a component of the sample design, since the samples of any state will retain their identity with that state, but serve rather as conveniently located receiving stations for serum analysis.

It is assumed that each veterinarian’s sample quota will be divided into sub-quotas for the various livestock types on which incidence of disease is deemed desirable. Consideration herein is given only to the two major livestock groups, cattle and swine.

* From the Biometrics Unit and the Veterinary Virus Research Institute, Cornell University, Ithaca, New York.
The survey will operate continuously on a more or less permanent basis, thus permitting periodic summarization and publication of estimates of disease incidence. Data will be summarized on a state, regional, and national basis. Individual state estimates are regarded as only of minor significance, except for those states producing a large fraction of the national population of a particular livestock animal. Interest, rather, is centered upon estimates for various agricultural regions and for the nation as a whole.

The relative number of serum samples of each livestock group taken from any state will be made to correspond roughly to the relative abundance of the animal in that state as determined by the latest (1950) census of agriculture. Since the probability of exposure to a disease increases with age of the animal, it might be considered necessary to provide estimates for separate age classes; in cattle this could be accomplished simply by eliminating calves from the study, while swine could be divided into the premarket and the breeder classes. Sample quotas for the two classes of swine would likewise be based upon their relative abundance according to the 1950 census. Proportional allocation of the sample assures that the most accurate estimates will be obtained for areas where the animal is most abundant and hence of greatest economic importance.

The actual number of serum samples to be collected is determined in part by cost consideration and in part by the level of accuracy required in the estimate of incidence. This problem was approached by first constructing a financially feasible plan and then examining its expected accuracy to determine any modifications that might be required to attain a degree of reliability which is reasonable in light of the purposes of the study. The seven regions corresponding to the seven receiving stations were formed in such a manner as to contain approximately the same number of head of livestock (including all cattle over three months of age and all swine), and it was estimated that each of the serology laboratories could conveniently handle up to 5,000 serum samples annually. A sampling plan was drawn up accordingly, allotting a total of 35,000 samples to the States in proportion to their 1950 livestock populations, and is exhibited in Table I.
<table>
<thead>
<tr>
<th>Region</th>
<th>State</th>
<th>1960 Census Cattle Over 3 Mo.</th>
<th>Swine All Ages</th>
<th>Proposed Sample Cattle Over 3 Mo.</th>
<th>Swine All Ages</th>
<th>Maximum Sampling Error at .95 Probability Level Cattle Over 3 Mo.</th>
<th>Swine All Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wash.</td>
<td>740,496</td>
<td>131,628</td>
<td>213</td>
<td>38</td>
<td>7%</td>
<td>16%</td>
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<td>Ore.</td>
<td>926,556</td>
<td>157,237</td>
<td>267</td>
<td>45</td>
<td>6%</td>
<td>15</td>
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<td>Calif.</td>
<td>2,392,508</td>
<td>538,812</td>
<td>690</td>
<td>155</td>
<td>4%</td>
<td>8</td>
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<td>Mont.</td>
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<td>105,961</td>
<td>440</td>
<td>30</td>
<td>5%</td>
<td>18</td>
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<td>Idaho</td>
<td>815,190</td>
<td>161,195</td>
<td>235</td>
<td>46</td>
<td>7%</td>
<td>15</td>
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<td>Wyo.</td>
<td>912,863</td>
<td>49,554</td>
<td>263</td>
<td>14</td>
<td>6%</td>
<td>27</td>
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<td></td>
<td>Colo.</td>
<td>1,542,590</td>
<td>283,199</td>
<td>445</td>
<td>21</td>
<td>5%</td>
<td>11</td>
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<td>N. Mex.</td>
<td>931,374</td>
<td>69,092</td>
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<td>20</td>
<td>6%</td>
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<td>Ariz.</td>
<td>569,854</td>
<td>27,931</td>
<td>164</td>
<td>8</td>
<td>8%</td>
<td>35</td>
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<td></td>
<td>Utah</td>
<td>494,888</td>
<td>71,742</td>
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<td>21</td>
<td>8%</td>
<td>22</td>
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<td>Nev.</td>
<td>377,315</td>
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<td>109</td>
<td>6</td>
<td>10%</td>
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<td>N. Dak.</td>
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<td>S. Dak.</td>
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<td>1,365,372</td>
<td>622</td>
<td>393</td>
<td>4%</td>
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<td>Nebr.</td>
<td>3,110,191</td>
<td>2,359,699</td>
<td>896</td>
<td>680</td>
<td>3%</td>
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<td>Kan.</td>
<td>2,965,003</td>
<td>1,288,994</td>
<td>854</td>
<td>371</td>
<td>3%</td>
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<td>Mo.</td>
<td>2,730,130</td>
<td>3,911,816</td>
<td>786</td>
<td>1,127</td>
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<td>956</td>
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<td>Ala.</td>
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<td>1,061,498</td>
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<td>306</td>
<td>6%</td>
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<td>Miss.</td>
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<td>Ark.</td>
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<td>Region</td>
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<td>3,710</td>
<td>1,551</td>
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<td>2.6</td>
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<td>Iowa</td>
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<td>53</td>
<td>8</td>
<td>14</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>N. H.</td>
<td>100,605</td>
<td>12,752</td>
<td>29</td>
<td>4</td>
<td>19</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Vt.</td>
<td>377,071</td>
<td>12,965</td>
<td>109</td>
<td>4</td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mass.</td>
<td>167,171</td>
<td>95,883</td>
<td>48</td>
<td>28</td>
<td>14</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>R. I.</td>
<td>22,550</td>
<td>6,504</td>
<td>6</td>
<td>2</td>
<td>38</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Conn.</td>
<td>152,446</td>
<td>25,246</td>
<td>44</td>
<td>7</td>
<td>15</td>
<td>38</td>
<td></td>
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<tr>
<td>Del.</td>
<td>52,733</td>
<td>38,839</td>
<td>15</td>
<td>11</td>
<td>27</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Md.</td>
<td>384,596</td>
<td>244,659</td>
<td>111</td>
<td>70</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>D. C.</td>
<td>180</td>
<td>1,365</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td>8,240,108</td>
<td>5,300,983</td>
<td>2,373</td>
<td>1,526</td>
<td>2.1</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>NATION</td>
<td>65,697,083</td>
<td>55,721,977</td>
<td>18,925</td>
<td>16,048</td>
<td>0.7</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>
The accuracy of such a plan may be appraised by examining the column headed "maximum sampling error at the .95 probability level." Figures in this column refer to the error in state estimates of incidence; their interpretation may be illustrated with the first figure for cattle for the State of Washington as follows: "The probability is at least .95 that the incidence in a random sample of size 213 will differ from the actual incidence in the State by less than seven percent." These calculations are based upon the assumption that a situation least favorable to estimation exists; namely, that the true incidence of disease is 50 percent. If the true incidence of a cattle disease in Washington is different from 50 percent then the probability of an error of estimate less than seven percent is actually greater than .95; hence, without knowing whether the true incidence will differ from 50 percent, we may state a priori that the probability of an error less than seven percent is at least .95.

The errors of estimates listed in Table I are perhaps too large to permit any practical use to be made of individual state estimates, at least for those states containing small livestock populations. Regional estimates, however, present a much more satisfactory picture, whether the regions correspond to those given here or to any other meaningful combination of states having common agricultural practices. In particular, estimates for the entire nation are of more than adequate accuracy for educational purposes. The appropriateness of a sample of 35,000, therefore, depends upon the relative importance assigned to state, regional, and national estimates, respectively.

The effect of varying the total sample size, either by reducing the number of receiving stations or the number of samples to be handled per station, is summarized for the national estimate in Table II. The total number of serum samples is reduced by steps of 5,000 from 35,000 to 15,000 and the corresponding errors of the national incidence estimates are listed. Table II may also be used for the purpose of determining the number of cattle or swine serum samples that must be tested in order to determine the national incidence of a disease to within any specified error. For example, if an experimenter wished to determine the national incidence of infectious bovine rhinotracheitis to within ± one percent he should test approximately 10,000 of the cattle serum samples collected in the national survey.

### TABLE II

*Error of the National Incidence Estimate from Various Numbers of Serum Samples*

<table>
<thead>
<tr>
<th>Cattle Over 3 Months</th>
<th>Swine All Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Serum Samples</strong></td>
<td><strong>Max. Error of Estimate</strong></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
</tr>
<tr>
<td>19,000</td>
<td>0.7</td>
</tr>
<tr>
<td>16,000</td>
<td>0.8</td>
</tr>
<tr>
<td>14,000</td>
<td>0.9</td>
</tr>
<tr>
<td>11,000</td>
<td>1.0</td>
</tr>
<tr>
<td>8,000</td>
<td>1.1</td>
</tr>
</tbody>
</table>
A preliminary cost analysis of the proposed sample design suggests that accurate knowledge of disease incidence could be obtained at a cost of slightly more than one dollar per serum sample. This appears to be a small price to pay for the many benefits to be derived from a survey such as this. In addition to aiding the evaluation of vaccination programs, disease incidence figures would objectively guide the development of combined vaccines, indicating for each region the combination of most prevalent diseases. Also, incidence figures would serve to inform veterinarians concerning the diseases occurring in their area and so enable them to make better diagnoses. Information on diseases common to both livestock and man would likewise aid the Public Health Service. The continuous record of disease incidence would provide valuable data for the study of epidemiology. Serum samples collected in this survey would be made available to laboratories as a serum reference bank to further promote the detection and control of animal diseases.
AMENDMENT TO THE CONSTITUTION AND BY-LAWS

DOCTOR HENDERSHOTT: The next order of business is the presentation of an amendment to the Constitution and By-Laws.

You will recall that at our meeting last year there was a proposed amendment to the Constitution and By-Laws offered, which was presented first at Chicago. It was acted upon favorably by the Executive Committee last evening, and according to our Constitution and By-Laws it must be presented to the Assembly at this time for final action.

The amendment reads as follows:

"Amend 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 government, the Territories, 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 or milk and meat hygiene, may be elected to individual membership."

Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4H Club member as well as future farmers under 21 years of age are eligible to election as junior nonvoting members.

The dues of nonvoting junior members shall be three dollars.

I would move, Mr. President, the adoption of this amendment to our Constitution and By-Laws.

DOCTOR KUTTLER: I second the motion.

Motion passed.
COMBINED VACCINES*

JAMES A. BAKER, D.V.M., PH.D.

Within the last decade, new techniques in the hands of trained veterinary scientists have led to the isolation and identification of a large number of viruses and bacteria which cause disease. After accurate serological techniques have been devised and described so that field cases can be identified, most diseases are found to be widespread, once the knowledge and means of identification also become widespread. A well known example of this is leptospirosis in cattle. *Leptospira pomona* alone is now recognized in nearly all parts of the United States as the cause of one of our most important cattle diseases. Even more recently, it is beginning to be recognized as responsible for a hitherto unsuspected disease in swine. Another well known example is the virus of infectious canine hepatitis, against which antibodies are found in more than 50 percent of all living dogs more than one year of age.

Veterinary scientists are dedicated to the principle that prevention of disease in animals is necessary for our animal economy. And they know that prevention of disease is better than a cure. On this thesis, veterinary scientists have applied new laboratory methods and have either modified in virulence or inactivated, or sometimes both, the majority of recognized disease producing agents that have been isolated. Of course, once such agents are demonstrated in a form suitable for vaccines, they can then be made articles of commerce.

Great technological advances have been made in vaccine production. Especially important has been the use of vacuum drying for preserving antigenicity of modified viruses. Both bacterial and viral agents thus modified in virulence but still living are generally recognized as producing quicker and longer lasting immunity than those inactivated. The live but modified vaccines produce immunity more nearly resembling that found in nature after recovery from the disease. Therefore, modified live agents are sought as the best immunizing vaccines.

As our knowledge continues to increase, the number of agents increases accordingly. Since these can, and should be made into additional live agent vaccines to prevent disease, we are faced with the possibility of a large number of inoculations if we continue to apply the principle that prevention is desirable. For obvious reasons, a large number of single vaccinations are not practical, thus leaving us with the only alternative of combined vaccines given as a single inoculation.

For many years, some producers of biological products have offered various combinations of inactivated bacterial vaccines. In some instances, bacterial vaccines have been combined with inactivated single viral vaccines. More

* From the Veterinary Virus Research Institute, Cornell University, Ithaca, New York.
recently, combinations of two different inactivated viral vaccines have been made. Still more recently, there have been made available vaccines that combine two live but modified viruses. All of these combined vaccines were designed to offer maximum protection with a minimum number of inoculations.

Combinations of live agents cannot be made as simple mixtures without careful study. In every case, several factors must be determined, the most important of which are safety and effectiveness. Each combination will first have to be proven safe and effective by laboratory and field tests. Then, after a combined vaccine meets the specification of safety and effectiveness, its worth should depend wholly and entirely on its contribution to efficient animal production.

Since combined vaccines, especially those containing live agents, are increasing in importance, vaccination will be reviewed and, by introducing for consideration a triple vaccine for cattle, something of future potential will be indicated.

THE MAKING OF A VACCINE

A vaccine does not come into being because a few people decided to make a product which they wish to sell for profit. Usually, it begins with a veterinary practitioner called to see sick animals. After several such episodes, a disease investigation is requested and research workers are assigned the task of locating the cause. Whenever the cause is found to be an infectious agent, especially but not necessarily a virus, another vaccine to prevent disease becomes a possibility.

In the past, etiological studies were often difficult. Today, with newer techniques, such as those of tissue culture for virus isolation, and with carefully designed buildings for studies of isolates in disease-free animals, a disease can be defined. The average loss it causes per animal can be evaluated and the findings can be related to field observations. After these and further studies, it becomes possible to devise a vaccine and test it accurately in order to meet the practical requirements of safety and potency suggested by Hejl (1).

Under present conditions, the need for vaccination is estimated and the demand created by animal producers is used to justify a decision to vaccinate. With the statistical method presented by Robson and Baker (2), however, incidence can be ascertained, vaccination needs can be determined, and predictions can be made of any given vaccine’s actual monetary value to our animal economy according to this formula:

\[
\text{total loss} \quad = \quad \text{incidence of the disease} \quad \times \quad \text{average loss per diseased animal} \quad \div \quad \text{total cost} \quad \div \quad \text{cost per vaccination}
\]
COST OF VACCINATION

Preliminary research on infectious diseases, although often costly and time-consuming, should be charged as additions to our sum total of basic knowledge and considered in a different category from development costs of a practical vaccine. Thereafter, these costs can be categorized as:

1. Cost for producing vaccine, which will include all expenditures for development, production, potency testing, sales, profit, etc.
2. Cost for administering vaccine is the sale price of the vaccine plus cost of its administration, including both the veterinarian's fee and the owner's labor costs for assembling and handling the animals to be vaccinated.

Combined vaccines emerge as a means of reducing expense. A combined vaccine provides immunity comparable to its individual components given singly. It should cost less to package and ship a mixture than the individual components singly. Potency testing cost of a combined vaccine should be no more than the total for tests of each component as a single vaccine, and it might be less. Most important, savings on labor costs of vaccination make it mandatory to use combinations, although single vaccines could be given simultaneously and not increase labor costs materially. However, there might evolve from combined vaccines a subtler economic gain based on increased usage. Whereas an owner might doubt the economic advantage in preventing the possibility of a single disease, he would recognize the economic value of a combined vaccine to give insurance against a multiplicity of diseases. He would be further encouraged to vaccinate if the difference in cost between single and combined vaccines is not too great. Indeed it should not be, since the only difference in the cost of vaccination against a single disease and with a combined vaccine would be the cost of the vaccine itself.

A COMBINED VACCINE FOR CATTLE

Poultry vaccines thus far have made use of combinations of live Newcastle virus and live infectious bronchitis virus to be given in single dosage form. A dual vaccine for dogs has been introduced in which both canine distemper and infectious canine hepatitis viruses have been modified. In each instance, there has been good reception of these combinations and, therefore, it seemed worth while to extend this idea to cattle vaccines, since even greater economic benefits could result when a combined vaccine was used for this animal.

Single vaccines against leptospirosis (3) and infectious bovine rhinotracheitis (4, 5) already have become articles of commerce, even though an accurate evaluation of dollar return to cattle production from their use is not known. Of course, this has not been possible, lacking full information on incidence, although these diseases have been established as widespread. For leptospirosis, some incidence figures (6, 7) are available and it is now recognized as a costly enzootic disease. Initially recognized as a feedlot problem, vaccine for infectious bovine rhinotracheitis has been used under these con-
ditions. As diagnosis has improved, this disease begins to assume enzootic characteristics similar to leptospirosis. Infectious bovine rhinotracheitis has been identified in 13 states, including the coast to coast States of California and New York. In New York, pooled serum samples from dairy herds showed five of 43 herds were positive (8), while a calf serum sample collected in 1941 in New Jersey contained serum neutralizing antibodies. This indicates that the disease has been occurring in cattle in the eastern United States for a long time. Virus diarrhea has been reported from Maine, New York (9), California (10), and Indiana (11). In New York, approximately half of the dairy calves are immune by six months, an indication of a high incidence rate.

In preparing a test combination (12), the modified live viruses of virus diarrhea and infectious bovine rhinotracheitis were dried together. A liquid inactivated type leptospiral vaccine was used for reconstitution. After vaccination, tests with virulent agents of each disease were given in sequence (Table I).

As Table I shows, each vaccinated calf was placed in contact with another known to be susceptible, as a safety control for the spread of vaccine viruses. Since the combined vaccine effectively immunized all vaccinated calves and the vaccine viruses did not spread to the controls, it was concluded that two of the requirements for a practical vaccine had been met in part. The vaccine was now ready for field tests.

**COMBINED VACCINES IN THE FUTURE**

Use of combined vaccines will depend on whether they contribute to efficient livestock production. Perhaps Herbert Schaller, editor of *Better Farming Methods*, had this in mind when he marked the observance of April, 1957, as Animal Health Month with the following editorial comment. "If we are to spotlight animal health and its role in agriculture, perhaps we ought to try and bring about some progress on the subject. Therefore, this suggestion on animal health might be in order: *Treat it positively rather than negatively.*

"Look at it this way. Farmers use fertilizer to boost yields. They do this as a positive management practice to give greater efficiency and try to increase profits. They don't wait until crops are growing and show signs of starvation before considering the use of fertilizer.

"Perhaps it is time to apply the same reasoning to animal health. Too many farmers take the opposite or negative approach to this management practice. They wait until something happens before applying sound animal health practices."

Vaccination as a means of increasing animal yield through disease prevention would appear to be one of the positive management practices called for by editor Schaller. The maximum profit from this practice would be realized if combined vaccines were used.

The triple vaccine for cattle described above is an example of the modern vaccines that can be made and tested in our veterinary laboratories today. With increasing facilities and better trained personnel, there is no limit, and
should be no limit to disease investigation. Disease must be prevented for profitable animal production and, with combined vaccines, disease can be prevented. No other procedure yet advanced has this potential.

REFERENCES


TABLE I

Tests for the Effectiveness and Safety of a Multiple Vaccine Given Simultaneously for Protection of Calves Against Virus Diarrhea (VD), Infectious Bovine Rhinotracheitis (IBR), and Leptospirosis (Lept.)

<table>
<thead>
<tr>
<th>Number of Calves</th>
<th>Clinical Response</th>
<th>Immunity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp.</td>
<td>Leuk.</td>
</tr>
<tr>
<td>6 (vaccinated)</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>6 (unvaccinated)</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* For tests, each vaccinated calf was placed in contact with another that was left unvaccinated as a safety control.
** Immunity determined by serological response to IBR and Lept. components and lack of illness following inoculations in sequence to virulent VD virus, then IBR virus, and finally L. pomona. Denominator indicates number of animals used and numerator those showing response to vaccination or evidence of immunity.
CHAIRMAN MILLIGAN: Thank you, Doctor Baker.
Are there any questions?

DR. A. K. KUTTLER [Salt Lake City]: We used to hear about one vaccine interfering with the development of immunity. I would like to hear what Doctor Baker has to say about that.

DOCTOR BAKER: I think you have hit the deterrent factor in the development of combination vaccines. Interference was very much studied, and much was written about interference by virologists in the last fifteen years. They would inoculate an animal with two viruses, and the animal would show symptoms of only one virus. The symptomatology produced by the other virus was suppressed. That was interference. Immunity was never considered.

I can find no instance in the literature, except for poliomyelitis, where the simultaneous administration of two agents would show an interference in the development of immunity against one or the other. In other words, interference relates to signs of disease, not to the development of immunity. In the case of poliomyelitis there is just one report in which, when the three types were given, one type did not establish itself.

VOICE: I would like to correct Doctor Baker. He said there is only one article in the literature, on Newcastle's disease and infectious bronchitis. The full significance of the serological results one gets is not fully known. It can be very clearly demonstrated that there is definitely a serological and probably also an immunological interference when the two are administered simultaneously versus individually.

DOCTOR BAKER: I take it the immunological has not been shown yet, since this vaccine is now used.

VOICE: It may be a quantitative rather than a qualitative factor.

DOCTOR BAKER: With immunity, of course, there is a quantitative factor. With viruses and live agents they are usually immune or are not immune.

DR. W. F. FISHER [Reno, Nevada]: Would you recommend adding to brucellosis vaccine, Blackleg and malignant edema bacterins?

DOCTOR BAKER: I think when we have single vaccines they must be studied in combination to see if they are or will be effective, because obviously we are not going to launch a vaccination program by offering the livestock producers a series of single vaccines. In fact, for example, brucellosis would never be commonly administered if it weren't subsidized.

I think if you took brucellosis and put in combination with brucellosis as many antigens as you can, without having any serious effect on the animal but deriving immunity, then you should do so, if that will increase animal production.
THE EVALUATION OF ANTHRAX SPORE VACCINE (NONENCAPSULATED) IN SHEEP


The incidence of anthrax in domestic livestock in the United States has remained relatively constant during the past 12 years. In the decade ending 1955, 3,569 separate outbreaks were reported, with a loss of nearly 18,000 animals (1). There are several widespread enzootic areas of infection in this country, and occasional epizootics such as occurred in Oklahoma during the summer of 1957 serve to emphasize the serious nature of anthrax to livestock raisers. Although the value of controlled immunization programs in livestock-raising areas has been proved (2), vaccination against anthrax in the United States has been done largely in a haphazard manner except in the face of epizootics. An important reason for this has been the known short comings of the various immunizing agents heretofore available in the United States: The Pasteur-type spore vaccines lack the margin of safety that is desirable when using live bacterial suspensions, while bacterins have not been good immunizing agents (4).

Sterne (5) (6) has reviewed the use of anthrax vaccines made from avirulent nonencapsulated strains derived as dissociants from smooth-mucoid strains of virulent organisms cultured on 50 percent serum agar in an atmosphere of high carbon dioxide content (7) (8). Personeus et al (9) reported the results of their studies on this type of anthrax vaccine in guinea pigs and sheep. Such vaccines, which utilize nonencapsulated variant strains of Bacillus anthracis, have a high margin of safety for most species of livestock, and have good immunizing properties.

Vaccines: Each cooperating laboratory produced two lots of vaccine essentially as described by Sutton (10) and Sterne (11). N-Z-amine (Sheffield Farms, Inc.), 1.0 percent, was substituted for the tryptic digest of casein; and Bacto yeast Extract (Difco), 0.5 percent, was used instead of a watery extract of dried brewer’s yeast. Lyophilized stocks of Bacillus anthracis strain 34F2, obtained from Dr. R. A. Alexander of Onderstepoort, Pretoria, South Africa, were prepared as suggested by Sutton (10). After harvesting, the concentrated suspensions were heated in a water bath at 60°C for 30 minutes to destroy vegetative cells. From this heated suspension, final vaccines were prepared in glycerin-saponin diluent (8) at a concentration of ± 2 x 10⁶ viable spores per ml. Guinea pig protection tests on each of these vaccine lots were satisfactory.

† Colorado Serum Company, Denver, Colorado.
**Animals:** Sixty young sheep were purchased through the Denver Union Stockyards and moved to a holding pen at the northern edge of the city. Adjustment to different environment and feed rations presented some problems during the first few days after admission. Three deaths occurred during the first three days of the holding period before vaccination, apparently from bloat caused by the addition of too much alfalfa to the ration. After correcting the feeding regimen, no further non-specific deaths occurred during the study. All of the sheep were wormed and held in quarantine for an additional 10 days before proceeding with the test.

**Experimental Procedure:** Each lot of vaccine was used to inoculate groups of 10 sheep. The dose was one ml administered subcutaneously at a clipped site on the side of the neck. The remaining 17 sheep were held as non-immunized controls; all animals were identified by appropriate metal ear tags. Immediately before vaccination and daily thereafter for the first 11 days, rectal temperatures of each vaccinated animal were taken. Through a misunderstanding, temperatures of the control sheep were not taken until the seventh day, but were recorded during the remainder of the period.

Thirty-one days later, all of the sheep were moved from the holding pens in Denver to a previously prepared site in the northwest corner of Douglas County, Colorado. The site was located roughly 35 miles southeast of downtown Denver, directly south of a large range area used by the Air Force for bombing and gunnery practice. The land was gently rolling and consisted primarily of sandy clay. The test site will be described and illustrated in greater detail later.

For challenge, nine of the control sheep and five animals from each vaccine subgroup were injected into the right axillary space with two ml of a spore suspension of *Bacillus anthracis* strain 99 containing approximately 30,000 spores per ml. The remaining vaccinees and controls were each given one ml of the same suspension. This challenge strain was a virulent culture received in 1949 from Fort Detrick, Frederick, Maryland; its virulence for sheep was not determined but repeated titrations have shown the MLD for guinea pigs to be approximately 600 spores. The challenge doses given, therefore, were approximately equivalent to 50 (1.0 ml) and 100 (2.0 ml) guinea pig MLD.

**RESULTS**

**Vaccination Responses:** Only two indications of postvaccination reactions were observed: First, in a few of the vaccinees, small subcutaneous lumps could be detected by palpation for the first few days after vaccination. None persisted for more than a week and no necrotic abscesses formed such as are occasionally observed following injection of diluents containing saponin into guinea pigs. One vaccinee exhibited lameness on the fifth day postvaccination, but was normal on the following day so nothing in the episode was attributed to the vaccination. Secondly, there was an apparent temperature rise in a majority of the vaccinees during the first 24-48 hours postvaccination. It is unfortunate that data from the control group is not available for
comparison, but the temperature rise observed on the first and second days probably was a specific response since it exceeded the variations observed on subsequent days. (see Figure 1.) Otherwise, the animals remained clinically healthy, ate well, and continued to gain normally.

Table I summarizes the results. Since there was no indication that any individual lot of vaccine performed differently from any of the others, all of the vaccine subgroups have been composited in this table.

**TABLE I**

Postchallenge Death Losses

<table>
<thead>
<tr>
<th>Sheep Group</th>
<th>Challenge Dose*</th>
<th>Number Challenged</th>
<th>Deaths, Hours Postchallenge</th>
<th>Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACCINEES</td>
<td>30,000</td>
<td>20</td>
<td>144, 168</td>
<td>10%</td>
</tr>
<tr>
<td>VACCINEES</td>
<td>60,000</td>
<td>20</td>
<td>72, 96</td>
<td>10%</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>30,000</td>
<td>8</td>
<td>48, 48, 56, 56, 72, 80, 96, 120</td>
<td>100%</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>60,000</td>
<td>9</td>
<td>48, 48, 48, 48, 72, 72, 72, 72, 72</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Spores of bacillus anthracis strain 90.

The course of infection, insofar as it was observed, was uniformly typical in all of the animals that succumbed. In the early clinical stages, the animals
assumed a position from which they were reluctant to move, as if there was pain in the joints. Anorexia followed or accompanied this sign. In a few of the animals a mucoid diarrhea was observed, but this was not a consistent finding. In all stages, a marked respiratory involvement was apparent and during the moribund stages a frothy, muco-hemorrhagic exudate was discharged from the nostrils of nearly every animal that died after the 48th hour. (Figure 2.) In one animal, a brief tetanic convulsion was seen. Rapid bloating of the carcasses was commonly observed.

Fig. 2. Frothy hemorrhagic exudate from mouth and nares of sheep in terminal stage of induced anthrax infection.

DISCUSSION

Anthrax spore vaccines prepared from nonencapsulated variants have been used in several species of livestock including cattle, horses, goats, camels and sheep (5) (6). Some horses experience a more pronounced reaction than do cattle or sheep (5), and occasional deaths have been attributed to vaccination of this species (12), although the accuracy of this attribution is open to question. Goats are reportedly less satisfactory subjects for vaccination, and South African workers advise caution in recommending the use of the non-encapsulated vaccines in such animals (11).

Thus, even the nonencapsulated type anthrax spore vaccines have certain shortcomings that somewhat limit their usefulness. Despite these shortcomings, however, it is felt that these vaccines represent a major improvement over the Pasteur-type spore vaccines commonly used in this country heretofore.

This study was not designed to determine how rapidly immunity to anthrax develops, nor how durable such immunity may be. Controlled challenge data regarding these factors have not been found in the literature.
Such data are extremely costly to obtain, particularly when one considers the precautions that must be taken when virulent challenge cultures are being used.

State and Federal livestock control officials, public health officers and livestock ranchers themselves are vitally interested in the precautions taken while conducting research such as has been described here, so a brief description of methodology follows:

First, permission to conduct the research in Colorado was obtained from the office of Dr. Gail Gilbert, who was interim State Veterinarian after the untimely passing of Dr. J. W. Childs. Authorization was then obtained from the Animal Inspection and Quarantine Division of the Agricultural Research Service under the provisions of Biological Products Memorandum 56-3.

As was mentioned previously, no special precautions were taken during the postvaccination observation period, but all phases of the challenge work were done in such a manner as to minimize the possibility of any spread of the virulent challenge culture used.

At the challenge test site, an excavation roughly 80' x 40' x 8' was made. At the bottom of this pit, a frame shed 40' x 11' x 7' was constructed, this being made insect-proof by the use of fine-mesh window screening. Padlocked doors were provided at each end. Nearby in the pit, a small shack was constructed in which all feed, tools, clothing and other equipment was kept. The entire pit was surrounded by two rings of five foot snow fencing, spaced approximately 10 feet apart; the access gates through these fences were padlocked and chained. Fences were put up to keep out stray cattle that might wander onto the tract and to discourage coyotes, rats or similar animals that might be attracted by the smell of dead sheep. (See Figures 3-5.)
Fig. 4. Another view of challenge test site, showing pen, auxiliary shed and holes used for carcass disposal.

Fig. 5. Interior of challenge pen. Fence separated vaccinees from controls to facilitate removal of carcasses.
Before entering any contaminated area of the test site, a nearly complete change of clothing was made by all persons. Coveralls, boots, caps, face masks, rubber gloves, and at times, cotton gloves, were worn whenever the pen was entered and whenever dead or infected sheep were handled. The process was reversed when leaving the premises, and in addition, a second change of clothing was made at an intermediate motel room, rented specifically for the purpose, before personnel resumed their normal activities.

Animals that died were removed as soon as found (at one of the twice daily visits to the test site) and were carefully lowered into four foot holes previously dug in the bottom of the pit. The carcasses were then saturated with kerosene and burned. While the carcasses were not completely consumed, all of the wool, exposed skin and external discharges were thoroughly incinerated, and this undoubtedly destroyed any anthrax spores that might have been present on such external surfaces. (Figures 6 and 7.) The charred remains were immediately covered with slaked lime, then buried with the original dirt from the hole.

Fig. 6. Contaminated carcasses were incinerated immediately following death.
Fig. 7. Charred carcasses after burning. These were immediately covered with lime and buried.

When the experiment was terminated, all of the surviving animals were shot and left within the pen. The entire area, including the carcasses, buildings, tools, clothing, boots—everything that had been used in the test pit—was saturated with kerosene and burned. (Figure 8.) Finally, the entire pit was back-filled with a bulldozer so that all remains of the test were buried from six to 10 feet below the surface of the ground. (Figure 9.)

Fig. 8. At termination of the study, the entire test site and all materials used were saturated with kerosene and burned.
SUMMARY

(1) Vaccination of 40 young sheep with preparations of anthrax vaccine, nonencapsulated type, resulted in a definite but transient temperature rise within 24 hours, but no other clinical effects were produced and no local abscesses or other persistent local reactions developed.

(2) One month postvaccination, 18/20 sheep survived challenge exposure to 30,000 (± 50 guinea pig MLD) spores of B. anthracis strain 99; 8/8 controls died within 120 hours. In a second group, 18/20 sheep survived exposure to 60,000 spores, while 9/9 controls died within 72 hours.

(3) A description of the methods used to minimize dissemination of the virulent challenge culture was given.

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REPORT OF COMMITTEE ON BIOLOGICS AND PHARMACEUTICALS


This report is a brief review of new biological, pharmaceutical and diagnostic agents that have been introduced and used in recent years in the management and control of diseases of livestock. Development and improvement of these agents have resulted from considerable research in educational, governmental, and industrial institutions and great progress is being made in increasing the production of livestock, improving feed efficiency, and controlling some animal diseases. Improved tissue culture techniques now used in the biological industry have made possible the development of several new prophylactic agents for use in animals.

INFECTIOUS BOVINE RHINOTRACHEITIS VACCINE

Infectious bovine rhinotracheitis, especially that afflicting beef cattle, has for several years aroused the concern of many livestock raisers, feeders, and veterinarians, and there have been several excellent studies of this disease. During the past year infectious bovine rhinotracheitis (IBR) vaccine has been produced by tissue culture techniques from modified live virus. Intramuscular administration is recommended for the vaccine.

Reports indicate (1) that the preparation is safe, (2) that the virus is not spread from vaccinated to nonvaccinated animals, (3) that vaccinated animals develop neutralizing antibodies against the infectious bovine rhinotracheitis virus, and (4) that vaccinated animals are resistant to experimental challenge with virulent virus and are protected against field exposure. Extensive clinical data have not yet been accumulated, but available reports on the product are encouraging.

INFECTIOUS CANINE HEPATITIS VACCINE

Infectious canine hepatitis vaccine of modified virulence is being produced by tissue culture technique and now is available commercially. This agent also has been combined with modified distemper virus prepared from chicken embryos and is used as a multiple or dual vaccine. In addition, a killed canine hepatitis virus of tissue culture origin also is available. Those concerned with the control of canine diseases have shown considerable interest in these preparations.
HOG CHOLERA ANTIBODY CONCENTRATE

Hog cholera antibody concentrate, a recently-developed biologic preparation, consists of hog cholera antibodies concentrated from serum by fractionation. The production of the serum used in this fractionation is, in general, controlled by the regulations applying to the production of regular anti-hog cholera serum. Regulations were modified to include the concentrate with other products that are subject to provisions of the marketing agreement and order regulating the handling of anti-hog cholera serum.

CLOSTRIDIUM PERFRINGES ANTITOXIN

_Clostridium perfringens_ antitoxin, Types B, C, and D, now is available as a combination antitoxin prepared from animals hyperimmunized with the major toxins of _C. perfringens_ that affect livestock. This new biologic preparation has considerable value if used early in treating an infection.

LEPTOSPIRA BACTERINS AND TEST ANTIGEN

Bacterins and test antigens continue to be useful in the effective control of leptospirosis in various species of animals. Serological data indicate that _L. pomona_ bacterin will protect cattle, swine, horses and sheep against infection. Bacterins containing _L. canicola_ and _L. icterohemorrhagia_ are also available.

The _L. pomona_ test antigen and techniques for using it, especially the rapid plate and capillary tube tests, appear satisfactory under field conditions.

ANTHRAX VACCINE

This past summer many thousands of cattle in Oklahoma were vaccinated with avirulent (Sterne type) anthrax vaccine. Favorable reports have confirmed those of the 1956 report regarding the American use of this type of vaccine. A report at the 1957 meeting will describe the experimental use of this vaccine in sheep. The experimental results undoubtedly interest many who are concerned with the problem of anthrax in animals.

AVIAN PLEURO-PNEUMONIA-LIKE ORGANISM (PPLO) INFECTION

Avian PPLO diagnostic antigen currently is produced under a special Federal license subject to the following conditions: "The licensee shall restrict the distribution and sale of the product to official state diagnostic laboratories, other reputable institutions and laboratories, and persons concerned with the research and diagnosis of poultry diseases who are qualified to interpret and evaluate the results obtained from the antigen." This antigen will be used primarily by research institutions and laboratories studying avian PPLO or air sac disease.

The diagnostic antigen is used in a serologic agglutination test to identify birds harboring the PPLO infection.
During the past year another effort was made to control PPLO infection in laying flocks by administering streptomycin or dihydrostreptomycin or a mixture of the two. Intramuscular or intraperitoneal administration of these substances to laying hens appears to increase egg production, improve hatchability and reduce morbidity in chicks hatched from eggs of treated birds.

**TREATMENT FOR LUNGWORM INFECTIONS**

Studies conducted in Great Britain indicate that cyanacethydrazide is useful in removing certain lungworms from cattle, sheep, and swine. Given subcutaneously or orally, the compound helps eliminate a large proportion of larvae and worms from the bronchioles, bronchi, and trachea. Although this agent does not kill the worms, it more or less narcotizes them and permits their expulsion. Treatment is effective only when there is no obstruction in the respiratory tract to prevent the ciliated mucous membrane from moving the worms up and out of the tract.

**HYGROMYCIN**

Hygromycin, a new antibiotic obtained from a strain of *Streptomyces hygroscopicus*, was discovered in a soil sample from Indiana. Identified first as an antimicrobial agent, it was recently found effective as an anthelmintic agent. The B fraction of the substance, especially, appears to possess prophylactic and therapeutic action against *Ascaris* (large roundworm), *Strongylus* (modular worm), and *Trichuris* (whipworm) infections in swine. Hygromycin drastically reduces the production of ova and has a lethal effect on these helminths.

It has been recommended that the broth from the cultured organism, including the mycelium, be added to carrier substance (as a premix) for addition to swine feed and that twelve million units of hygromycin B be added to a ton of starter and grower rations for young pigs.

**DOW-ET-57**

*(0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate)*

An organic phosphorus compound identified generally in reports of experimental studies as ET-57 has been shown to be effective in the control of the migrating larvae of cattle grubs, *Hypoderma bovis* and *Hypoderma lineatum*, when it is administered orally in doses of 100 milligrams per kilogram of body weight. It is effective in killing the migrating larvae up to and including the third instar larval stage in cattle. At this dosage there appears to be no visible toxic effect in the host, yet it kills 70 to 100 percent of the parasites. Somewhat larger doses (150-200 mg./kg.) have produced evidence of temporary toxic effect. There is some evidence that the compound may be stored in fat tissue in treated animals. The recommendations are that it not be given within 60 days of slaughter, and under no circumstances to dairy cows.
The compound bears further study in the control of external parasites, such as lice, fleas, flies, and mites.

Recently Herlich\(^1\) reported that the drug also possesses anthelmintic activity against certain gastrointestinal nematodes in cattle.

TRANQUILIZING DRUGS

A group of relatively new compounds, used clinically in animals during the past few years, are of primary interest in this country for the treatment of mental diseases in humans. Desirable characteristics, especially their “chemical restraint” effect, have brought the tranquilizing drugs into wide use in both large and small animals. These agents have greatly facilitated the handling of large animals, which otherwise would be very difficult to treat effectively and there is some evidence that they may reduce stress factors related to shipping fever.

Although many studies are in progress to determine other uses for the tranquilizing drugs, it would be premature to predict all indications for their use in livestock.

THE USE OF PENICILLIN G IN CATTLE BLOAT

Because the occurrence of bloat is of major significance to the cattle industry, the problem is receiving considerable attention from various investigators. Several recent reports indicated that antibiotic substances fed or administered orally were effective in reducing the incidence of bloat, especially when cattle were feeding on certain legume pastures. Given in 25-75 mg. doses, both the procaine and potassium salts of penicillin G are of value in mitigating the incidence of bloat in cattle fed on ladino clover pasture. The drug should be administered several hours before the animals are exposed to the hazard of bloat.\(^2\)

IRON THERAPY

Hypochromic anemia occurs frequently in young animals, especially in pigs. Various oral dosage forms of iron have been used, and recently an injectable iron-dextran complex became available for intramuscular administration. Each two ml. of this dosage form yields 100 mg. of elemental iron. This substance is especially useful for controlling anemia in baby pigs, for one injection will provide normal iron requirements for 50 to 60 days.


STANDARDS FOR VETERINARY BIOLOGICS

The 1956 report of the Committee on Biologics and Pharmaceuticals mentioned that the Veterinary Biological Licensees Association assisted in the development of and recommended minimum temporary requirements for the production of ovine ecthyma vaccine, wart vaccine, encephalomyelitis vaccine, feline distemper vaccine, canine distemper vaccine, infectious canine hepatitis vaccine, rabies vaccine, and hog cholera vaccine.

Over the past year the results of these tentative recommendations have been encouraging, and with slight modifications they have now been accepted as the minimum requirement. One major revision applying to rabies vaccine (modified live virus, chick embryo origin) gives licensees the alternative of using fixed rabies virus for challenging potency-test animals instead of using canine salivary-gland virulent street virus. The fixed virus has been found satisfactory and eliminates the hazards to the laboratory worker from street virus.

Recently, the Animal Inspection and Quarantine Division put into effect a "Redirection of Inspection," which will provide a more accelerated and uniform method of inspecting all veterinary biologics. A "Manual of Inspection Procedures for Biological Products" has been furnished to all AIQ veterinarians engaged in such work. Work conferences were held at Indianapolis, Kansas City, and Omaha with all inspectors who will have a part in this program. The manual was discussed thoroughly so that the redirection program would be initiated in a uniform manner.

A MANUAL OF STANDARD PROCEDURES FOR USE IN TESTING VIRUS VACCINES

A subcommittee of the Inter-Regional Committee on Respiratory Diseases of Poultry consisting of representatives from State Agricultural Experiment Stations and the Federal agencies currently are preparing a manual describing "Standard Procedures for use in testing Virus Vaccines for use in Poultry."

It is our opinion that this manual will be quite useful to those engaged in poultry disease control work.
A REPORT ON RESEARCH ACTIVITIES IN ANAPLASMOSIS

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There has been an increased interest in the disease, anaplasmosis, in recent years. I will point out only the highlights of the research activities on this disease. I wish to indicate that part of the material presented this afternoon was taken from papers presented at the Third National Research Conference on Anaplasmosis in Cattle at Kansas State College, June 12-13, 1957.

The following states or agencies have reported work currently in progress on anaplasmosis. In alphabetical order, they are: University of California, University of Florida, Kansas State College, Louisiana State University, Montana State College, University of Nevada, Oregon State College, Oklahoma State University, University of Pennsylvania, University of Tennessee, Texas A. & M. College, and the Agricultural Research Service, United States Department of Agriculture. The most intensive research is on the following phases: (a) study of the nature of the etiological agent, (b) studies on the complement-fixation test, (c) studies on the use of antibiotics.

It is appropriate that I define anaplasmosis before proceeding with the recent research activities on this disease. Anaplasmosis is an infectious disease of cattle and sheep caused by Anaplasma marginale and Anaplasma ovis and is characterized by progressive anemia due to destruction of red blood cells. Anaplasmosis in sheep appears in a much milder form than in cattle. The extent of this infection is not known at the present time. Anaplasmosis marginale has been reported by Saulmon (1) in 38 of the 48 States. The classification is still controversial, but it is the opinion of many workers to leave it in its present classification until conclusive evidence has been presented for its proper classification.

CHARACTERISTICS OF ANAPLASMA MARGINALE

Mott (2) reported that he and co-workers have made the following observations and studies of the Anaplasma marginale organism (parasite). The parasite increases in size after invading the red blood cell, and the number of bodies will vary from one to seven. This number is in direct proportion to the percentage of red cells which contain bodies. They have calculated that the average life of an infected red cell containing large marginal bodies to be three to four days. They have also been able to calculate quite accurately what the red cell count or degree of anemia will be three days in advance by checking the percentage of red cells infected. Some workers (3) have apparently proven that these marginale bodies break into numerous smaller bodies, and this occurs at the time that the red cell disintegrates. They also reported an increase in the size of anaplasma after the invasion of red

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blood cells. It then undergoes division, resulting in production of eight small spherical bodies in each anaplasma.

Recent studies (4, 5) with the electron microscope reveal that the parasite, in the mature stage is composed of a central undivided mass and peripheral rounded bodies of high electron density of a size below the limit of resolution of the optical microscope. The occurrence of these submicroscopic units may represent stages in the developmental cycle of this parasite within the red cell and also help to explain why the blood of carriers remains infective when no anaplasma can be found or recognized with certainty.

Recent findings by Ristic (6) indicate that *Anaplasma marginale* may be detected by means of fluorescein labeled antibody. Further work is required before a definite statement can be made as to the accuracy of detecting the carrier state in this disease with this technique. Splitter et al. (7) found that *A. ovis* and *A. marginale* exhibit cross antigenicity by the anaplasma complement-fixation test. Cattle cannot be infected with *A. ovis* but goats were found susceptible to this species.

**INCUBATION PERIOD**

The average incubation period has been found to be between 14 and 45 days in experimental animals. Mott (2) has observed one case to be 60 days. No doubt, we should expect the incubation period for natural field cases to be of similar duration or longer. Additional studies are required of the life cycle during the incubation period. There is evidence that the infectious agent is present in the red blood cell when no marginal bodies are present. A toxic agent is observed in the blood in acute cases. This apparently is the result of a protein decomposition product resulting from destruction of the red blood cells. Similar reactions are seen in blood transfusion with other anemias. The first attack of marginal bodies in an animal is always the most severe. Many animals, if they survive, will show a second attack in two or three weeks. In a few animals, these mild attacks may be observed up to one year or longer. It is believed that these attacks are a part of the life cycle similar to malaria infections.

**TRANSMISSION**

The disease is spread by biting or blood-sucking insects, bleeding needles, dehorning instruments, castration knives, etc. Mechanical transmission in most instances can be controlled by proper aseptic procedures.

Howell (8) has reported that nineteen species of ticks belonging to seven genera have been incriminated in the spread of anaplasmosis. He further indicated that nine species of horseflies are capable of transmitting this disease. Mosquitoes may also serve as vectors, although Howell did not consider them to be an important factor in the spread of the disease. He also indicated that deerfly had been incriminated but were not important, as only a limited number of transmissions had proved successful. Stableflies and hornflies have not been proven to be incriminated in the spread of anaplasmosis. Some arthropods may serve as mechanical vectors while others may be biological
vectors. In a few cases, ticks may serve as both biological and mechanical vectors. Mechanical transmission is possible for short periods only, probably not more than five minutes after the vector has fed, whereas biological vectors feeding on acute cases may spread the disease for years, and during this period may be carried for long distances.

THE CARRIER ANIMAL

The carrier animal is one that no longer shows marginal bodies in the red blood cells, but the infectious agent, though obscure, is still apparently present in the red blood cell. Most animals that have had an acute attack of the disease will remain carriers of the disease for life, but there have been a limited number of known cases which lost their carrier infection and again became susceptible. Mott (2) has proven that the infectivity titer ascends with and gradually drops along with the serum antibody titer after the initial disease attack. Similar results have been obtained in Kansas in A. ovis infection in sheep.

STUDIES ON THE COMPLEMENT-FIXATION TEST

It is apparent that since the first report of the complement-fixation test for anaplasmosis by Mohler (9) and Rees and Mohler (10), the test has become very accurate and reliable. Mott and Gates (11) and Price et al. (12) have improved the test by improving methods in the production of the antigen. A report was given by Price et al. (13) as to practical application of the complement-fixation test for anaplasmosis before this group in 1953. Willers (14) has reported that in his opinion the complement-fixation test is sufficiently accurate and will serve as a tool for the eradication of anaplasmosis under Hawaiian conditions. They have tested 147,114 head of cattle with .43 percent reactors. Spliter et al. (15), in a survey of anaplasmosis in cattle, indicated that the outlook appeared promising for eventual control of this disease in Kansas. Roby (16) reported the eradication of anaplasmosis in a beef herd of 165 head over a four-year period by means of the complement-fixation test and a program of segregation and disposal. Other field trials by Sanders (17), Tunnicliff (18), and Saulmon (19) show favorable reports on limited numbers with the employment of the complement-fixation test and disposal of reactors. In Kansas, veterinarians are requesting the complement-fixation test as an aid in the control of the disease.

We can conclude that the test, therefore, is very efficient in detecting carrier cases of anaplasmosis. A small percentage of false positive and false negative reactors are known to occur with this test. I believe that sufficient data (Roby 20) are available to indicate that this test is 98 percent or more accurate.

There is a need for the development and recognition of an official diagnostic test for anaplasmosis. Such a test could be used for incidence surveys, field control programs, imports, exports, and interstate movement, as well as for better comparative studies by research workers at various stations. I am sure that most of us remember from past experience the difficulty encountered with the standardization of the brucellosis test in its inception. In order to avoid
making a similar mistake with anaplasmosis testing, a need exists for early recognition, study, and agreement concerning a method for standardization of the complement-fixation test for anaplasmosis.

STUDIES ON THE USE OF ANTIBIOTICS

Many drugs have been tested against this disease since it was first recognized, according to Hagan and Bruner (21), by Theiler in 1910. However, none were found effective until Foote et al. (22) in 1951 demonstrated that chlortetracycline had anaplasma-inhibitory activity. This work was substantiated by Miller et al. (23), Foote and Wulf (24), Foote (25), Pearson and Brock (26), Splitter and Miller (27), Brock et al. (28, 29), and Splitter and Anthony (30).

Miller et al. (23) and Splitter and Miller (27) reported that oxytetracycline possessed definite anaplasma inhibitory properties. Brock et al. (31), Foote (32), and Pearson et al. (33) reported that tetracycline possessed a depressant action on the anaplasma agent.

Pearson (34) has reported that .5 mg. per pound prevented anaplasmosis from developing or the carrier state of the disease.

It is economically prohibitive in routine practice to use these antibiotics for treatment, as Foote (25) has reported the cost to vary from 40 to 60 dollars per 1,000-pound animal, the cost depending upon the route of administration. It is hoped that a less expensive drug can be found in the not too distant future.

CONCLUSION

I believe that with the present knowledge available, anaplasmosis could be eradicated from our tick-free areas and possibly even from tick-infested areas with an all-out effort.

I wish to advise that a motion was made and passed at the Third National Research Conference on Anaplasmosis in Cattle in June, 1957, to appoint a committee to draw up an outline for a "Standardized Complement-Fixation Test for Anaplasmosis." The group felt that there was a real need for the development and recognition of an official diagnostic test for anaplasmosis. "This test is to be used for incidence surveys, field control programs, import, export, and interstate movement, as well as for a better comparative study by research workers."

The committee was in agreement that the anaplasmosis test standardization should include a uniformity of testing materials, testing methods, and testing interpretation in order to obtain uniform results by different laboratories.

The committee, therefore, recommended that the antigen amboceptor, complement, and control sera be produced, standardized, packed, and distributed from one central control agency to assure uniformity of testing materials, and that the hemolytic amboceptor be approved only by this agency. This central control agency should be the United States Department of Agriculture, Animal Disease Eradication Division, in accordance with administrative direction by the office of the Agriculture Research Service.
REFERENCES

Anaplasmosis is today a disease which still remains an enigma in veterinary medicine. It is a disease whose causative agent is not well known and whose pathogenesis is unknown. It is a disease which, until very recently, was treated only empirically except in those cases where the symptomatic treatment of blood transfusion could be used. It is a disease for which man has yet to develop a workable system of prophylaxis but one, in enzootic areas, where nature has by producing carrier immunity at an early age. It is a disease which has strained practitioner client relationships due to our inability to rationally minimize or control heavy losses in severe range outbreaks. It is a disease which has cost the cattle industry millions of dollars and one which is now commanding our continuous and coordinated interest in basic research and investigation.

I practice in Santa Barbara County in California where I also serve as part time county livestock inspector. This area has considerable mountain and foothill country whose only use is to pasture range beef cattle. This range is covered with sagebrush to a large extent, and this brush is heavily infected with ticks. These ticks, due to transovarian passage of the infection (1) and by being infected from carrier animals, are apparently quite solidly infected with *Anaplasma marginale*.

A good amount of the beef cattle industry is seasonal. Rains begin about Thanksgiving and terminate about May 15th. Probably 40 or 50 thousand cattle are imported each fall to pasture the resulting green grass, and then are shipped to feed lots about June 15 when the grass has died due to lack of moisture. These imported cattle come from many parts of the United States. Many have not been previously exposed to anaplasmosis and the result can be, at times, a considerable incidence of anaplasmosis in our area. Many local cattlemen have become intimately familiar with the disease. As a result, in recent years, we have observed a preponderence of young weaner calves being imported in order to avoid the acute and fatal form of the disease as is usually found in older animals. Other cattlemen who operate native breeding herds have a low incidence of the disease except occasionally in newly purchased breeding bulls from Anaplasmosis free areas and in cows who somehow escaped early calfhood infection. Recent investigations on one of these ranches, in cooperation with the University of California (2), revealed that 95 percent of the animals tested had achieved carrier immunity by the time they were 18 months old when they would have begun to develop the acute and peracute forms of the disease.

As with many diseases, the clinical symptoms of anaplasmosis can be classified as being mild, acute, and peracute. First, however, there are five
cardinal symptoms which, when found together, usually spell anaplasmosis and only anaplasmosis. They are:

1. anemia.
2. weakness.
3. a febrile reaction.
4. grossly normal urine.
5. constipation.

Second, we usually find the following:

1. icterus.
2. inappetance.
3. depression.
4. dehydration.
5. labored respiration.
6. irrational behaviour.

Third, we must note that clinically it is a disease which is generally mild in calves up to one year of age, acute but not often fatal in cattle up to two years of age, acute and often fatal in cattle up to three years of age, and often peracute and often fatal in cattle over three years of age.

Fourth, it is clinically important to note that no weak and very anemic animal can tolerate much physical exertion. Treatment requires at least some restraint. Therefore, the prognosis is always much more unfavorable in a wild range animal than in the average farm animal who is accustomed to being handled by man.

Differential diagnosis of anaplasmosis is usually not difficult. It is readily differentiated by laboratory means. Clinically, although it manifests the anemia, weakness, and icterus of many of the contagious bovine anemias, it can be rather accurately diagnosed by the grossly normal urine and the presence of constipation no matter how green and lush the forage. The relative age of the animal is also important clinically. In the bovine anemias found in our practice other than anaplasmosis, the acute cases are found in the younger animals while the older group manifest either mild or subclinical symptoms.

On post-mortem, in anaplasmosis, anemia and or icterus are evident, the kidneys and urine are grossly normal, but the spleen is greatly enlarged as is the gall bladder which usually contains a thick sometimes even granular like bile. Moreover, the petechiation of organs found in many of the septicemias is often absent. In leptospirosis, on the other hand, we find hemoglobinuria in addition to the splenomagaly, icterus, and anemia. The kidneys are petechiated, the liver is sometimes a gingerbread consistency while the gall bladder and bile are relatively normal. In bacillary hemoglobinuria caused by Clostridium hemolyticum, a pathognomonic liver infarct is reported to be present (3). Generally speaking, then, the acute contagious anemias will present hemoglobinuria except anaplasmosis and, in our practice anyway, only anaplasmosis manifests constipation.
Now, after having briefly reviewed the pertinent differential symptomatology, we must visualize a herd outbreak of anaplasmosis in order to appreciate its vicious destructiveness. The picture I present has been repeated many times in our area, and it is a syndrome we dread to face.

Several years ago, a man purchased a ranch in our area and moved his entire herd of Hereford cows down from an anaplasmosis-free section of northern California. About 90 days after he arrived, he called to say that three cows had mysteriously died in the past week and that he had just discovered one that was very ill. On arriving at the ranch, I was asked to go on to the pasture as the riders were unable to bring the animal in. I went out to find a large eigh-year-old Hereford cow holding two riders at bay. She was standing with head held high, pawing the ground, and turning from time to time to face one rider then the other. She appeared completely irrational and definitely “on the fight.” Her whole body was trembling and rigid, and her respiration was deep and labored. In her nervousness, she was constantly dripping small amounts of normal-looking urine and passed some hard mucus-covered stools. Her udder was icteric and numerous ticks could be seen on her body.

Suddenly she broke away and ran with a stilted staggering gait. I told the riders not to follow. She ran about 60 yards then stopped to turn and face us. In about a minute, she suddenly lay down. The riders approached her and she attempted to regain her feet but was unable to do so before she was roped. A quick hemoglobin reading was taken, a glance revealed a pure white vaginal mucus membrane and she was treated while struggling continuously. Within three minutes, she was released and we left her. She continued struggling, finally staggered to her feet, lurched about 30 yards when her forelegs collapsed beneath her and she fell. When we approached, she was in a tetanic convulsion with her eyes rolled back and her mouth open struggling for air. Suddenly she relaxed, and was dead.

Two days later, another case was found. She was treated and died. A day later, another case developed but we were prepared with whole citrated blood. She died before she had received 300 cc. The next day, another case was found and she received one gallon of whole blood. She never regained her feet. Four days later, another case died during the transfusion. The next day another case collapsed and died before she could be roped. A week went by before we had another case. She was treated with drugs and died that night. Meanwhile three others had simply been found dead. In the next 90 days, the man lost 30 more cows, some of which were treated and most of which were not. He didn’t feel I was doing them much good, and I agreed.

Another ranch nearby has probably lost 250 shipped in cows in the past 10 years. A third ranch in our hottest anaplasmosis belt often lost between 50 and 60 shipped in cows per season. Another rancher once told me he could have purchased a nice cattle ranch with the money anaplasmosis has cost him in his lifetime.

We have now described what I call the peracute form of the disease. We also have an acute form which is most common in our native cattle. Here
we have an animal who is usually not belligerent, and who may be driven slowly to a corral although she may rest occasionally along the way. Her hemoglobin may be below four grams per 100 ml blood, her stools are mucus-covered pellets, and she can easily be exerted enough to kill her. But she will usually not be viciously uncooperative and kill herself. Her prognosis is quite good with modern treatments in the early clinical stage, and with even whole blood if it is available. Nearly all of our ranchers have from two to a dozen of these cases annually in native cattle, and they are apparently animals who escaped becoming carriers at an early age.

Then there is a mild form. About three years ago, I examined a weaner calf for a client but could find very little wrong. The stool was normal as was the hemoglobin but there was a slight fever. A blood slide revealed some anaplasma bodies. No treatment was given and the calf recovered. Later, the owner informed me that nearly the entire group of 50 calves revealed some weakness and depression at some time during the next 90 days. However, I am informed these animals can die if violently exerted as during branding, castrating, and vaccinating (4). I have personally seen a six months old calf with the acute form who had a blood count of 1,350,000 red cells and a hemoglobin reading of 2.7 grams per 100 ml of blood. Without much question then, we have considerable overlapping of our three classifications in different age groups, and our experience indicates that clinical recovery is very much dependent on hemopoietic response which appears to be much more quickly attained in the young.

Treatment of anaplasmosis is difficult especially in acute and peracute cases. We have felt our treatments were largely empirical until the development of the tetracycline drugs in recent years. In our practice, we have used oxytetracycline or terramycin and found that, unlike many drugs used previously, it did have an aborting clinical effect on the disease when used in the early clinical phase. At this time, a minimum dosage of three mg per pound of body weight would frequently produce a definite clinical improvement within 24 to 48 hours. However, it was interesting to note little change in the hemoglobin picture until about the fourth day. This suggested that by inhibiting the organism, we might be inhibiting some toxic agent produced by the organism, or we might be lessening blood cell breakdown and an auto-intoxication due possibly to an excess of blood breakdown constituents in the body (5). Again, after inhibiting the organism, rapidity of recovery appears dependent on the capacity and rapidity of hemopoietic response.

Too often, however, we deal with a herd outbreak in which therapy is always too late. We are forced then to take whatever prophylactic steps are available. Stauffer, in 1947, reported the prophylactic use of sodium caco-dylate in modifying the clinical phase of the disease (6). We have found this drug often wanting therapeutically but prophylactically it has given us some beneficial, or at least lucky, results. On several outbreaks, we have administered it to the balance of the herd and terminated the development of further clinical cases. In one outbreak, however, we noted no particularly beneficial results. The rationale of its use in this way may be in its pre-
clinical stimulation of hemopoiesis. Workers at the University of California have, I understand, aborted the clinical phase of the disease with oxytetracycline injections in some experimental animals (7). Workers in Oklahoma have had similar results in a feed lot with chlorotetracycline orally (8). The use of the tetracyclines in this manner, if properly developed, appears to us a most logical and rational approach.

A clinical report to you on this disease should include an expression of the client's attitude in an enzootic area. We have many men in the cattle industry who have an abundance of experience with the disease from the very important economic point of view. They are also well informed with its clinical picture and realize that, in enzootic areas, many adult cattle would not be alive today if nature's vectors and possibly some of their own surgical errors had not provided a carrier immunity early in the life of each animal. With a constant reservoir of infection being maintained in our tick population and possibly in our wild life, they feel that some form of immunity is absolutely essential for survival of the adult animal in many areas of our country. My experiences with anaplasmosis force me to strongly concur with this point of view.

We hope then that work in the near future will isolate and reveal the classification of Anaplasma marginale. We hope that its nature is such that it can be modified to retain its antigenicity and lower its infectivity. Detection and elimination of carriers is not in itself, we feel, the answer to the problem in many enzootic areas. Instead, we view with great interest attempts like those of Oklahoma workers to develop some form of vaccine for the disease (9). For it appears to use at this time, that this will be the only tool with which we will ultimately completely control this disease in many of our nation's great beef cattle ranges.

BIBLIOGRAPHY

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REPORT OF COMMITTEE ON ANAPLASMOSIS

K. J. Peterson, Chairman, Salem, Oregon; J. Christensen, Davis, California; J. A. Henderson, Fort Worth, Texas; J. A. King, Phoenix, Arizona; W. T. Oglesby, Baton Rouge, Louisiana; L. J. Poelma, College Park, Maryland; T. O. Roby, Silver Spring, Maryland; E. H. Willers, Honolulu, Hawaii.

Your Anaplasmosis Committee is happy to report that very definite progress has been made during the past five years in the fight against anaplasmosis. It appears that through the use of the complement-fixation test to identify carrier animals and the slaughter of these carrier animals, the eradication program being presently carried on in the Territory of Hawaii is nearing completion. Dr. Ernest H. Willers, Territorial Veterinarian, states that only one dairy herd remains which has not had at least one clean test and this herd had only one reactor on its last test, which was completed in September, 1957. Doctor Willers further states, “In my opinion, the CF test is a good tool in an eradication program under conditions similar to those existing in Hawaii.” This eradication program, which is a cooperative one between the Territory of Hawaii and the Agricultural Research Service, United States Department of Agriculture, not only has been of great benefit to the livestock industry of Hawaii, but also has practically proven that the disease can be eliminated from areas where:

1. The disease is not firmly entrenched.
2. The insect vectors are of the type that transmit the disease by mechanical means.
3. Sprays are used to assist in controlling the insect vector.
4. The ranchers and veterinarians are cognizant of their role in the possible spread of the disease and take all precautionary methods necessary in keeping surgical instruments, vaccine and bleeding needles, tattooing equipment, etc., clean and aseptic.
5. A sound, well administered regulatory program is maintained.

Your Committee is of the opinion that the Hawaiian officials and the ARS should be complimented on the eradication program they are conducting in Hawaii. The benefits to the livestock producer and the knowledge gained in the control of anaplasmosis under field conditions, has been well worth the funds expended by the United States Department of Agriculture in this project.

The eradication of this disease from a number of herds in the east and southeast by the same method as used in Hawaii, that is the test and slaughter method, gives new hope for the control and eradication of the disease from certain areas of the United States proper. In these areas the vectors, primarily the tabanids, are mechanical spreaders as were the vectors in
Hawaii. A spraying program to control these vectors aids considerably in the eradication program.

Dr. L. J. Poelma, Chief of Laboratories, Maryland State Board of Agriculture, states, "The incidence of clinical anaplasmosis in Maryland appears to be on the decline for the last two years. Whether this was brought about by more intensive use of insect sprays in problematical. We do notice that the insect population in general has been greatly reduced since some of the towns and counties have carried out extensive spraying programs during the summer months. Such a program used on livestock might materially reduce the incidence of anaplasmosis."

While progress is being made in the control and eradication of the disease in certain areas, in other areas anaplasmosis continued to spread and continues to cause heavy losses. Little, apparently, has been done to halt the progress of the disease where the insect vector, the tick, a biological carrier rather than a mechanical carrier, is prevalent. There is no known practical method of eradicating the ticks, especially in the large range areas of the West. The etiological agent may be transmitted through the egg of the ticks from one generation to the next and may remain in the ticks for as long as four to five years from the time the initial tick fed on an infected animal. These infected ticks are capable of transmitting the disease to cattle when they again have an opportunity to feed on the blood of these animals. The slaughter of immune carrier cattle from ranges where there is an abundance of infected ticks does not seem practical since clean replacement cattle could readily become infected via the tick reservoir. A vast area of intermountain
range country, including a part or all of each of the 11 western states, is affected by tick-borne anaplasmosis. The cost to the producers in this area from this disease certainly runs into millions of dollars annually. More research in an effort to control and eradicate the disease in this great area would be justifiable.

To determine whether or not the test and slaughter plan or a test and segregation plan could be used successfully in such an area, your committee recommends that the ARS conduct a program on a heavily infected ranch, located in the range area where ticks are abundant. Vector control measures with insecticides should be used in conjunction with the programs, as well as any new methods of treatment or control which may be developed. If such programs prove both successful and practical, eradication of anaplasmosis from the United States would be possible.

Your Committee also wishes to report that the third National Research Conference on Anaplasmosis was held at Kansas State College on June 12 and 13, 1957. Seventy-seven registered for the meeting, in contrast to 39 at the meeting held in 1953. Twenty-three states, Puerto Rico, Hawaii and Mexico were represented. Twenty very interesting research papers were presented and a panel on the "standardization of the Complement-Fixation Test" was held. These research papers clearly indicated that there is, at present, a considerable amount of excellent research being conducted throughout a large area of the United States. These papers dealt with the nature, distribution, epizoology, transmission, diagnosis and treatment of anaplasmosis. The research included the use of tissue cultures and the electron microscope. Florescent antibody studies and studies of the metabolism of radio active iron in anaplasmosis were also conducted. These papers indicate that the most modern methods and techniques are being used in this research and it is almost a certainty that if this interest in anaplasmosis research is maintained, new important developments which will assist in the control, treatment and eradication of anaplasmosis will be forthcoming.

As indicated by Dr. M. J. Twiehaus in the paper which he has presented at this meeting, a committee for the standardization of the anaplasmosis complement-fixation test was appointed at the third National Conference for Anaplasmosis. This Committee has met several times and through considerable effort and time on the part of its members has developed a standard procedure for the test. This recommended procedure is too detailed and lengthy to read at this time. However, it will be included in the printed report of your Committee on Anaplasmosis. Your Committee on Anaplasmosis recommends that this procedure be adopted in order to obtain a uniform and standardized test. The test is to be used for field diagnosis and control programs, for import and export shipments, and further research studies.

To properly conduct this test a supply of standard antigen and complement must be available for both regulatory and research work. Therefore, your Committee on Anaplasmosis further recommends that funds be appropriated to the Animal Disease Eradication Division of A.R.S. for the continuing production of anaplasmosis complement-fixing antigen and complement.
RECOMMENDED PROCEDURE FOR A STANDARDIZED COMPLEMENT-FIXATION TEST FOR ANAPLASMOSIS*

I. General Information

A. The Antigen - Antibody - Complement System.

1. The antigen consists of the antigenic fraction of bovine lysed erythrocytes collected from an acute experimental case of anaplasmosis. The antigen is diluted with veronal buffer solution adjusted to a pH of 7.3 to 7.4 and employed in the test at two antigenic units based on titration with standard positive serum.

* The original procedure was prepared by the Animal Disease Eradication Division, Agricultural Research Service, U.S. Department of Agriculture.
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2. Clear, phenolized, non-hemolyzed serum of a suspected animal is tested for the presence of specific antibody. Inactivated serum (58°C for 35 minutes) is diluted in veronal buffer solution (0.1 ml. serum plus 0.4 ml. veronal buffer) for the test. Serum should be obtained from blood samples which have been drawn under aseptic conditions and every precaution should be taken to avoid hemolysis.

3. Normal guinea pig serum is used as complement. It is diluted in veronal buffer solution so that each 0.5 ml. contains two exact units (minimum amount required to produce complete hemolysis).

B. The Hemolytic System consists of complement as described in #3 above, plus 1.0 ml. of equal parts 2 percent washed sheep red blood cells and hemolytic amboceptor (anti-sheep hemolysin) containing two units per 0.5 ml. Amboceptor and sheep cells are mixed in equal parts and held at 37°C. ten minutes before using.

C. The total volume of reagents in this test is 2.5 ml. Pyrex test tubes 15 x 85 mm. without lips, therefore, should be used in this procedure. A stainless steel tube rack, army medical type, with two rows of holes (ten each) is recommended.

II. Detailed Outline of the Procedure for the Complement-Fixation Test

A. Reagents.

1. Veronal buffer (Isotonic) stock solution.
   a. Preparation.
      NaCl (C.P.) ...................................... 85.0 g.
      5,5 diethylbarbituric Acid ...................... 5.75 g.
      Sodium 5,5 diethylbarbiturate ................ 3.75 g.
      Distilled water—q.s. .......................... 2,000 cc.
      Sterilize at 15 lbs. pressure for 15 minutes.

      Dilute one part of the above stock solution with four parts distilled water the day used pH 7.3-7.4
   b. Used as follows:
      (1) Serves as a diluent (1 to 4 dilution) throughout the test for serum, antigen, complement, amboceptor, and sheep cells.

2. Alsever’s Fluid.
   a. Preparation.
      Sodium citrate ... 12.0 grams.
      Sodium chloride ... 4.2 grams.
      Dextrose .......... 20.5 grams.
      Distilled water ... 1,000 ml.
      (1) The sodium citrate and sodium chloride are dissolved in 800 ml. distilled water and sterilized at 15 lbs. pressure for 15 minutes. Dissolve the dextrose in 200 ml. distilled water, and sterilize either by filtration or autoclaving. Add aseptically to the sterile saline-citrate solution.
      (2) Dispense aseptically 150 ml. of the sterile solution into 500 ml. Erlenmeyer flasks which are marked at the 300 ml. level.
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      (1) A normal adult sheep is bled from the jugular vein into a 500 ml. flask containing 150 ml. of Alsever's fluid (blood collected using aseptic precautions).
      (2) When sufficient blood is collected to raise the level in the flask to the 300 ml. mark, the needle is then withdrawn from the vein, and the site is swabbed with 70 per cent alcohol.
      (3) The blood and preserving fluid are mixed thoroughly and stored at 4°C. in the refrigerator. This is the stock supply of sheep red blood cells, and it keeps satisfactorily for a period of two weeks.

   b. Saline Suspension of Sheep Red Blood Cells.
      (1) Each day that sheep cells are required, the contents of the flask containing the stock are thoroughly mixed by gentle rotation.
      (2) Sufficient blood to meet test requirements is aseptically removed by means of a sterile pipette and placed in a round bottom centrifuge tube.
      (3) Centrifuge for 10 minutes at 2,000 r.p.m. I.E.C.* Centrifuge No. 1 at 2,000 r.p.m.—Centrifuge No. 2 at 1,700 r.p.m.
      (4) Decant supernatant fluid by suction through a capillary pipette, taking off upper white layer of cells. Refill tube with veronal buffer diluent and resuspend cells by inverting and gentle shaking of the tube.
      (5) Centrifuge again for 10 minutes at 2,000 r.p.m.
      (6) Decant the veronal buffer solution and repeat washing two more times, totaling three washes of cells in veronal buffer solution. The cells should be transferred to a 15 ml. graduated and tapered centrifuge tube for the third washing.
      (7) Suspend the packed cells in veronal buffer solution according to the reading on the centrifuge tube to make a 2 per cent suspension which is used in the test.

   c. Fragility Test for Red Blood Cells.
      (1) If supernatent fluid is not colorless on third washing, cells are too fragile and should not be used. Fresh sheep blood should be collected.

4. Amboceptor.
   a. Commercially produced amboceptor approved by the Animal Disease Eradication Division (A.D.E.D.), Agricultural Research Service, will be used.
   b. Two units of amboceptor are used in the test. The amboceptor is diluted in veronal buffer solution so that this quantity is contained in 0.5 ml. (See III-B.)

* International Equipment Company, Boston, Massachusetts.
5. Antigen.
   a. Antigen supplied and standardized by the A.D.E.D. is used in the
      C.F. test. (See III-C.)
   b. The antigen is stored at -30°C. to -70°C. in a dry ice chest
      (preferably -70°C.).
   c. Antigen is diluted with veronal buffer so that 0.5 ml. contains two
      units. (Undiluted antigen not used in test may be refrozen at -30°C.)

III. Standardization of Components of the Complement-Fixation Test.

A. Complement titration.

1. A 4 percent solution of complement is prepared (adding 0.5 ml. of
   complement supplied by A.D.E.D. to exactly 12.0 ml. of veronal
   buffer). The stock complement stored at -30°C. to -70°C. (preferably
   -70°C.).

2. Procedure.
   The protocol shown in Table 1 is followed. When the proper
   amounts of all components have been added, the rack is then placed
   in a water bath at 37°C. for 45 minutes. The test is immediately
   read at the end of the incubation period.

<table>
<thead>
<tr>
<th>Tube Numbers</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer – ml.</td>
<td>1.45</td>
<td>1.425</td>
<td>1.4</td>
<td>1.375</td>
<td>1.35</td>
<td>1.325</td>
<td>1.3</td>
<td>1.275</td>
<td>1.250</td>
<td>1.5</td>
</tr>
<tr>
<td>4 percent complement – ml.</td>
<td>0.05</td>
<td>0.075</td>
<td>0.10</td>
<td>0.125</td>
<td>0.15</td>
<td>0.175</td>
<td>0.20</td>
<td>0.225</td>
<td>0.250</td>
<td>—</td>
</tr>
<tr>
<td>Amboceptor – 2.0 units/0.5 ml.</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2 percent cells – ml.</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The exact unit is the smallest amount of diluted complement (4 percent)
showing complete and sparkling hemolysis in 45 minutes at 37°C. Complement
is then diluted in veronal buffer so that two exact units are
contained in a 0.5 ml. volume. The following equation is used to de-
termine the amount of veronal buffer necessary to add to guinea pigs’
serum (complement) so that each 0.5 ml. contains 2.0 exact units.

25 (dilution of complement titrated) : (dilution to use)
two units 0.5 ml. (Quantity containing
two units)

For example, if the unit in Table 1 is read at tube number 5 (0.15 ml.
of 4 per cent complement)

25 : X

2x0.15 0.5
solving: X equals 41.6

Thus, 1.0 ml. of complement (A.D.E.D.) plus 40.6 ml. veronal buffer
would contain two exact units of complement per 0.5 ml.
B. Amboceptor (Antisheep hemolysin) titration.

1. A 1:100 and 1:1000 dilution of amboceptor is prepared:
   a. Add 0.1 ml. antisheep hemolysin to 9.9 ml. of veronal buffer.
   b. Add 1.0 ml. of the above 1:100 dilution to 9.0 ml. veronal buffer.

2. Complement (A.D.E.D.) is diluted so that 2.0 exact units are contained in 0.5 ml. (See previous calculations under III—"Procedure.")

3. The protocol shown in Table 2 is followed for further amboceptor (antisheep hemolysin) dilutions.

### TABLE 2

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Hemolysin</th>
<th>Veronal Buffer</th>
<th>Final Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 ml. of 1:1000 plus</td>
<td>1.0 ml.</td>
<td>1:2000</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ml. of 1:1000 plus</td>
<td>2.0 ml.</td>
<td>1:3000</td>
</tr>
<tr>
<td>3</td>
<td>1.0 ml. of 1:1000 plus</td>
<td>3.0 ml.</td>
<td>1:4000</td>
</tr>
<tr>
<td>4</td>
<td>1.0 ml. of 1:1000 plus</td>
<td>4.0 ml.</td>
<td>1:5000</td>
</tr>
<tr>
<td>5</td>
<td>1.0 ml. of 1:3000 plus</td>
<td>1.0 ml.</td>
<td>1:6000</td>
</tr>
<tr>
<td>6</td>
<td>1.0 ml. of 1:4000 plus</td>
<td>1.0 ml.</td>
<td>1:8000</td>
</tr>
<tr>
<td>7</td>
<td>1.0 ml. of 1:5000 plus</td>
<td>1.0 ml.</td>
<td>1:10,000</td>
</tr>
<tr>
<td>8</td>
<td>1.0 ml. of 1:6000 plus</td>
<td>1.0 ml.</td>
<td>1:12,000</td>
</tr>
<tr>
<td>9</td>
<td>1.0 ml. of 1:8000 plus</td>
<td>1.0 ml.</td>
<td>1:16,000</td>
</tr>
<tr>
<td>10</td>
<td>1.0 ml. of 1:10,000 plus</td>
<td>1.0 ml.</td>
<td>1:20,000</td>
</tr>
</tbody>
</table>

After the dilutions described in Table 2 have been made, 0.5 ml. of each dilution, starting with the preliminary dilution of 1:1000, is transferred to each of 11 tubes set up as shown on Table 3. After addition of complement (two units per 0.5 ml.) and 2 percent sheep cells, the rack is placed in the 37°C. water bath, incubated for 45 minutes, and read immediately.
TABLE 3

Titration of Hemolysin

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Hemolysin</th>
<th>Complement</th>
<th>Buffer</th>
<th>2 percent sheep cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 ml. of each</td>
<td>2.0 exact units</td>
<td>ml.</td>
<td>ml.</td>
</tr>
<tr>
<td>1</td>
<td>1:1000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1:2000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>1:3000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>1:4000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>1:5000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>1:6000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>1:8000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>1:10,000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>1:12,000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>1:16,000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>1:20,000</td>
<td>0.5 ml.</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>none</td>
<td>0.5 ml.</td>
<td>1.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The unit of amboceptor is defined as the smallest amount of diluted hemolysin to cause complete and sparkling hemolysis of 0.5 ml. of a 2 percent sheep cell suspension in 45 minutes at 37°C. in the presence of two exact units of complement.

Example of calculation:

If the last tube showing complete and sparkling hemolysis at the end of the incubation period is tube #6, a 1:6000 dilution (Table 3), this is the exact unit. Two exact units would then be tube number 3, a 1:3000 dilution of hemolysin (0.5 ml. contains two exact units).

C. Antigen Titration.

1. Reagents required to check the antigenic unit of antigen:
   a. Antigen diluted in veronal buffer to the approximate range of activity.
   b. Complement diluted in veronal buffer to contain 2.0 exact units in 0.5 ml.
   c. Amboceptor diluted with veronal buffer to contain 2.0 exact units in 0.5 ml.
   d. Sheep erythrocytes diluted to a 2 percent suspension with veronal buffer.
   e. Inactivated standard positive and standard negative sera. (Known negative and positive sera supplied by A.D.E.D.).
The antigenic unit is defined as the smallest amount of diluted antigen which will completely fix (4+ reaction) two units of complement in the presence of the standard positive serum. The anticomplementary unit is the amount of diluted antigen which inhibits hemolysis in the presence of standard negative serum.

2. Procedure.

The protocol as shown in Table 4 is set up for antigen titration. To each tube is added the amounts of veronal buffer, inactivated standard serum, antigen and complement as shown in the table. The rack is shaken to insure thorough mixing and placed in the 37°C water bath for one hour. During this incubation period, the 2 percent sheep cell suspension is mixed with an equal quantity of hemolysin containing two units per 0.5 ml. This mixture is then sensitized in the 37°C water bath for 10 minutes prior to use. One ml. of the warm mixture (sensitized red blood cells) is added to each tube and the rack is again shaken and returned to the water bath for 45 minutes. The rack is removed and results read immediately at the end of the incubation period.

<table>
<thead>
<tr>
<th>Front Row of Tubes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.875</td>
<td>0.85</td>
<td>0.8</td>
<td>0.75</td>
<td>0.70</td>
<td>0.65</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Antigen</td>
<td>0.025</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
<td>0.25</td>
<td>0.4</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Positive serum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Complement</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Amboceptor &amp; R.B.C.</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Incubate 1 hr.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>in water bath</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rear Row of Tubes</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.85</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td>0.9</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Antigen</td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Neg. Serum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>—</td>
<td>0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Complement</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Amboceptor &amp; R.B.C.</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Incubate 37°C. 45 min.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

3. Interpretation of titration.

a. Controls

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>9. positive serum control</td>
<td>hemolysis</td>
</tr>
<tr>
<td>16. antigen control</td>
<td>variable hemolysis</td>
</tr>
<tr>
<td>17. negative serum control</td>
<td>hemolysis</td>
</tr>
<tr>
<td>18. complement control</td>
<td>hemolysis</td>
</tr>
<tr>
<td>19. amboceptor and red blood cell control</td>
<td>no hemolysis</td>
</tr>
</tbody>
</table>
In tubes one through eight we should observe the spectrum of hemolytic activity from complete hemolysis to complete fixation (complete fixation somewhere between tubes two and five (0.05 to .2 ml.). If complete fixation is observed in every tube containing positive serum except in the positive serum control (tube nine), evidently the antigenic unit is stronger than the chosen dilution of antigen, and a higher dilution is tried until the range from complete hemolysis to fixation (4+) is obtained in the test. Thus, if the first tube showing complete fixation (4+) is number 3, the unit is 0.1 ml. of the diluted antigen. To calculate the dilution of antigen to contain two exact units per 0.5 ml., use the following proportion:

\[
\frac{\text{dilution of antigen}}{2 \text{ units}} = \frac{X}{\text{volume of test dose (0.5 ml.)}}
\]

For example, if the antigen had been diluted 1:6 and after titration the first tube indicating complete fixation was 0.1 ml., then:

\[
\frac{6}{2 \times 0.1} = \frac{X}{0.5 \text{ ml.}}
\]

Solving: \( X = 15 \)

Thus, the antigen would be diluted one part plus 14 parts buffer to contain two units per 0.5 ml.

It has been observed that many antigens are anticomplementary when tested along with the hemolytic system. However, if they are not anticomplementary in the presence of negative serum (tubes 10 through 15), the antigen is considered satisfactory for use in the test proper. A titration for hemolytic properties of the antigen alone is not routinely performed as anaplasmosis antigens have never given evidence of hemolytic qualities. Some antigens are observed to be moderately turbid and thus do not afford a sparkling hemolysis even when all the blood cells are lysed. In such cases, extreme care must be given at the time of reading in order to distinguish between turbidity due to the antigen from that due to the hemolyzed blood cells.

IV. The Test Proper

A. To perform the test proper, standardization of all reagents must be accomplished prior to use. These reagents and their values are as follows:

1. Antigen diluted in veronal buffer. Test dose is two exact units contained in 0.5 ml.
2. Complement diluted in veronal buffer to contain 2.0 exact units in 0.5 ml.
3. Amboceptor diluted in veronal buffer to contain 2.0 exact units in 0.5 ml.
4. Sheep erythrocytes diluted to 2 percent concentration in veronal buffer.
5. Standard positive and standard negative serum (A.D.E.D.) for controls.

B. Procedure.
For every serum being tested, 0.1 ml. is pipetted in each of two tubes containing buffer, one of which serves as the serum control. The tubes are then placed in a water bath at 58°C. for 35 minutes. This operation serves to remove from the serum any complement native to the serum, as well as to destroy any anticomplementary factors present. Remove the rack from the water and allow the inactivated serums to stand at room temperature for 90 minutes. The protocol for the test proper is shown in Table 5. The rack is removed from the water bath and read at the end of the 45 minutes incubation, or it may be placed in the refrigerator at 4-6°C. overnight and read the next morning.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Tube 1</th>
<th>Tube 2 (serum control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.4 ml.</td>
<td>0.9 ml.</td>
</tr>
<tr>
<td>Test serum</td>
<td>0.1 ml.</td>
<td>0.1 ml.</td>
</tr>
<tr>
<td>Antigen – 2 units</td>
<td>0.5 ml.</td>
<td>–</td>
</tr>
<tr>
<td>Complement – 2 units</td>
<td>0.5 ml.</td>
<td>0.5 ml.</td>
</tr>
<tr>
<td>Amboceptor &amp; Red Blood Cell</td>
<td>1.0 ml.</td>
<td>1.0 ml.</td>
</tr>
</tbody>
</table>

C. Interpretation of the Test Proper.
All serum controls, those tubes which did not receive antigen, should be completely hemolyzed. If any serum control tube contains un-hemolyzed cells, the sample is reported “anticomplementary” and no further interpretation is made as to the status of the animal’s serum. It is necessary to obtain another sample within 30 to 60 days on the animal. Those tubes which contain the test samples plus antigen may show reactions from complete hemolysis (negative reaction) to complete fixation (positive reaction). The interpretation of those reactions following between these two points is as follows:

- (4 plus) no hemolysis .......................... Positive
- (3 plus) 25 percent hemolysis .................. Suspicious
- (2 plus) 50 percent hemolysis .................. Suspicious
- (1 plus) 75 percent hemolysis .................. Suspicious
- (trace) only few cells remaining .............. Negative
- Complete hemolysis .............................. Negative
V. Serum Titration (Antibody Level Determination)

The titer of a positive serum is indicative of the stage of the disease whether it is from an acute or carrier animal. This is accomplished by serially diluting the serum, starting with a 1:5 dilution, in buffer until a final dilution of 1:1280 is attained. The reagents specified under the "Test Proper" are exactly the same except the test serum is diluted 1:5 by adding 1.0 ml. of the serum to 4.0 ml. of the veronal buffer. This 1:5 dilution is then inactivated by placing it in the 58°C. water bath for 35 minutes. Twelve tubes are placed in a rack and labeled according to the protocol in Table 6. Tubes one and 10 are left empty; to the other tubes is added 0.5 ml. veronal buffer. One-half ml. of the inactivated serum is added to tubes one, two and 10. Thus, tube number one contains 0.5 ml. of a 1:5 dilution as well as the serum control, number 10. Tube number two now contains 1.0 ml. of a 1:10 dilution. After thorough mixing 0.5 ml. is accurately pipetted from tube number two and placed in tube number three. This serial dilution process is repeated until tube number nine contains 1.0 ml. of a 1:1280 dilution; 0.5 ml. of which is removed and discarded. All tubes now contain 0.5 ml. of the respective dilutions. The standardized reagents are added in the order shown in Table 6.

<table>
<thead>
<tr>
<th>Tube Numbers</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1:5</td>
<td>1:10</td>
<td>1:20</td>
<td>1:40</td>
<td>1:80</td>
<td>1:160</td>
<td>1:320</td>
<td>1:640</td>
<td>1:1280</td>
<td>1:5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veronal buffer</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Antigen (2 units)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complement (2.0 units)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Incubate 37°C. — 60 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amboceptor (2.0 units)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2 percent sheep cells</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Incubate 37°C. — 45 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Interpretation of the Serum Titration

The serum titer is defined at the highest dilution showing a complete fixation (4 plus) reaction. Thus, if tubes one through five had 4 plus reactions and tube six gave a 2 plus reaction, the titer of the serum would be 1:80. The controls should read as follows:

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>hemolysis</td>
</tr>
<tr>
<td>11</td>
<td>hemolysis</td>
</tr>
<tr>
<td>12</td>
<td>no hemolysis</td>
</tr>
</tbody>
</table>

Each time titrations of unknown sera are performed, the standard positive serum having a known antibody titer should be included as a control.

DISCUSSION—ANAPLASMOSIS

Doctor Peterson [continuing]: Doctor Twiehaus has left a number of copies of the recommended procedure for the standard complement-fixation test for anaplasmosis on the table here. It is too lengthy to read, but if you are interested and wish to take a copy with you they are available up here.

Thank you, Mr. President.

President Good: Thank you very much, Doctor Peterson.

Is there any discussion of this disease?

Dr. L. E. Foote [Baton Rouge, Louisiana]: We are starting some work in Jeanerette, Louisiana. The samples are sent to Washington and the test is run there. These are precursory and preliminary tests, but we have some information that indicates that the complement-fixation test is quite erratic in this herd. There are 10 to 12 animals in the herd that are not running according to Hoyle, so to speak.

First, may I say that we started testing this herd last fall, a year ago. The herd was tested again last spring, again last summer. Every year the herd will be tested three times.

Last fall one of the cows was a reactor to the test. She became negative to the test last spring and this summer. The other cow was negative to the test last fall and also last spring. She was positive to the test this past summer.

I have challenged those two cows with 30 cc. of virulent blood. Both developed anaplasmosis, one the para-acute type and the other between the mild and the acute type.

In this herd we have a bull. That animal was negative to last fall’s test. He was positive to the summer test. Two weeks later another serum sample was taken from the bull and was negative.

We are doing some subinoculation studies on some of these cows, especially those with this erratic history on the complement-fixation test, and this bull was placed on experiment by using two splenectomized susceptible anaplasmosis calves. The one calf was born last May, and after developing a mild form of eperythrozoonosis it has come down with anaplasmosis. The
other calf just recently showed eperythrozoonosis, and I expect that calf to develop anaplasmosis; perhaps it has done so since I left home a few days ago.

I have six experimental steers at Baton Rouge that I inoculated from an animal that is a carrier of anaplasmosis and eperythrozoonosis. These six steers developed acute anaplasmosis. Several complement-fixation tests following their sickness showed all these animals to carry high titers; that is, they were reactors to the complement-fixation test. The last test, which was done in September, showed one of the animals now to be negative, four of them to be suspicious, and one to be a reactor.

I don't know what the story is going to be. We are going to follow this work further. In other words, we are going to sub-inoculate from animals that have been consistent reactors. I just thought I ought to present this information at this time, Mr. President, because the complement-fixation test is certainly erratic in our experience.

I don't know for certain, but it appears to me that the thing that is clouding the picture is this eperythrozoonosis. There have been two or three occasions when cattle in this herd in Jeanerette have developed anaplasmosis. Slides have been sent to us, and we have found both anaplasmosis and eperythrozoonosis to be current infections in the same cow at the same time.

I think you all know there is an interference phenomenon that exists between eperythrozoonosis and anaplasmosis. I don't know if it is a case of blocking out the anaplasmosis that is done by eperythrozoonosis clouding this picture or not, but I think it is very interesting.
PRELIMINARY RESULTS WITH A WHOLE-BLOOD AGGLUTINATION TEST FOR BOVINE BRUCELLOSIS

PAUL BOULANGER, D.M.V., M.S., and A. N. SMITH, D.V.M.*

The development of an economical and effective procedure to presumptively identify brucella-infected cattle becomes increasingly important as an eradication program advances. The standard tube and plate agglutination tests have been valuable aids in brucellosis control work carried out in Canadian "listed herds." But as soon as such control programs are extended on a country-wide basis, the need for a more convenient, time- and labor-saving method becomes imperative. Such a need is especially felt in the case of range cattle. Here, what is called for is a rapid method of diagnosis which will furnish results at the first handling of the animal and so avoid keeping "negative" cattle in the corrals for several days.

In dairy cattle, the development of the "milk ring test" (ABR) by Fleischhauer (1) in 1937, and its improvement to its present level of sensitivity by the Danish and Swedish workers (2-7), has assisted greatly in brucellosis eradication programs in many countries. However, in a country like Canada where there is a seasonal production of milk, the ring test has somewhat limited value since reactor animals may not be in production at the time the tests are being made.

Obviously with range and beef cattle, the milk ring test is of no assistance. In 1935, Welch and Marsh (8), experimented with a whole-blood field test for range cattle using citrated blood. As antigen they employed a suspension of Brucella abortus organisms stained with methyl violet. In 1941, Marsh (9), described certain modifications in the preparation of stained Br. abortus antigen for the whole blood test. In view of the obvious need for a test that would be accurate and yet easy to apply in the field, particularly with range cattle, it seemed well worthwhile to investigate further the value of the whole blood plate test. Preliminary work soon indicated that certain improvements were needed before such a method could be considered satisfactory for diagnostic purposes. Such improvements will be described in the present report.

METHODS

Preparation of Antigen

In preliminary trials, the Br. abortus antigen was prepared in various concentrations and stained with different amounts of crystal violet or Giemsa stains. The pH of the antigen and salt concentration were also varied. Different concentrations of sodium citrate as anticoagulant were added to the antigen. The antigen finally adopted was a 4 percent suspension of Br. abortus organisms stained with hematoxylin and suspended in physiological saline at pH 4.0 to 4.3. United States Bureau strain No. 1119-3, received from Dr. E. L. Love, was used in the preparation of the antigen for the field tests described in this paper. Comparative field tests are now being made with antigens prepared by Dr. A. Girard of our Animal Diseases Research Institute, Hull, Quebec.

*Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.
PRELIMINARY RESULTS FOR BOVINE BRUCELLOSIS

Institute with the Canadian Br. abortus strain, No. 413, which is used in our standard tube-agglutination test antigen. Various culture media are also being compared as to their suitability for antigen production.

In brief, the technique for preparation of the antigen for whole blood tests has been the same as for the milk ring test antigen with the exception that the anticoagulant heparin is added to the final product. The amount of heparin incorporated in each milliliter of antigen was 80 to 160 international units.

Technique of Test

The animals were bled either from the ear vein with a specially designed stylet or from the tip of the tail with a curved scalpel blade (Figures I, II, and III). A drop of non-clotted blood was taken with a wire loop, 5 mm. in diameter, made from 0.8 mm. gauge wire. The blood was mixed with 0.03 ml. of the hematoxylin-stained antigen delivered with a dropper on a glass plate and incubated at 35-37°C. over a heat-controlled box especially designed for the purpose (Figure IV). The readings were made at various intervals after mixing blood and antigen. With recently-infected or vaccinated animals, agglutination appeared almost immediately after mixing. With blood from cases of old infection, agglutination developed more slowly and was sometimes not perceptible until just before the blood dried on the plate, that is in about 10 to 15 minutes after mixing. Agglutination was still plainly readable and characteristic even after the blood had dried on the plate (Fig. V).

Fig. I. Collection of a drop of blood from the ear-vein of a cow with a stylet and a wire-loop.
Fig. II. Collection of a drop of blood from the tip of the tail of a cow with a specifically designed curved scalpel blade and a loop.

Fig. III. The stylet used for puncture of the ear-vein and the scalpel blade used in cutting the tip of the tail together with the attached wire-loop.
PRELIMINARY RESULTS FOR BOVINE BRUCELLOSIS

Fig. IV. The incubation-box with its thermostatically-controlled heat attachment and its moveable support.

Fig. V. A whole-blood agglutination reaction with a negative control.
Selection of Animals for Testing

Forty-one herds, containing 990 animals, were selected and tested by this whole blood method and the results compared with those of the standard tube and plate serum agglutination tests. Twenty of the herds, with a total of 496 animals, were known to be heavily infected. The other 21 herds, made up of 494 animals, were considered to be either entirely negative herds or herds containing only a few animals showing "questionable" reactions as a result of vaccination.

RESULTS

A comparison of the results obtained in the plate and tube serum agglutination tests with those of the whole blood test on 352 non-vaccinated animals is made in Table I. In this table the reactions have been divided into three groups according to the diagnostic interpretation they would receive if considered individually on the basis of the tube agglutination results. The negative group includes all sera that gave either an entirely negative reaction in tube agglutination tests or a reaction in the 1:25 dilution only; 282 of the 352 sera fell into this negative group. With the whole blood test, 18.1 percent of the 282 gave agglutination reaction of varying degree. That is to say,
81.9 percent of the cattle could have been safely sorted out as negative on the basis of the whole blood test alone. The remaining 18.1 percent reacting in the whole blood test would need further testing by the standard tube serum agglutination test before being judged as negative.

In the second group are included sera that gave questionable tube test results; that is, they reacted in the 1:50 dilution in this test. Ten animals fell in the questionable category. Nine, or 90 percent of them reacted in the whole blood test. Only one animal gave a trace reaction that might possibly have been overlooked had it not been in a herd containing eight other animals that reacted strongly in all tests. In the third group are included 60 animals that reacted positively, that is in a 1:100 dilution or higher. All 60 of these, or 100 percent, also reacted in the whole blood test.

Table II gives the results obtained with 638 vaccinated cattle. In the negative group are included all vaccinated animals that were non-reactive or

<table>
<thead>
<tr>
<th>Serum agglutination titers</th>
<th>Number tested</th>
<th>Number under 3 years</th>
<th>Whole blood reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number tested</td>
<td>Number under 3 years</td>
<td>Neg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Negative group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg. to trace 1:50</td>
<td>299</td>
<td>55</td>
<td>239</td>
</tr>
<tr>
<td>1:50</td>
<td>205</td>
<td>35</td>
<td>120</td>
</tr>
<tr>
<td>1:100</td>
<td>41</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>1:200</td>
<td>53</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Questionable group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:100</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Positive group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:200</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Table II**

*Comparison of the Results of the Serum Tube and Plate Agglutination Tests for Brucellosis with those of the Whole Blood Test in Vaccinated Cattle*

<table>
<thead>
<tr>
<th>Serum agglutination titers</th>
<th>Number tested</th>
<th>Number under 3 years</th>
<th>Whole blood reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number tested</td>
<td>Number under 3 years</td>
<td>Neg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Negative group</strong></td>
<td>605</td>
<td>107</td>
<td>79.0% (13.3%)</td>
</tr>
<tr>
<td>Questionable group</td>
<td>14</td>
<td>3</td>
<td>0. %</td>
</tr>
<tr>
<td>Positive group</td>
<td>19</td>
<td>2</td>
<td>0. %</td>
</tr>
</tbody>
</table>

*Number given in brackets indicate the percentage of vaccinated animals under 3 years of age included in the particular reaction group.
that did not react beyond the 1:50 dilution; 605 of the 638 vaccinates fell into this negative group. Of these 605, 21.0 percent showed agglutination in the whole blood test which would have necessitated further testing by the standard tube test. However, this percentage would have been reduced to 16.8 percent had no vaccinated animals under three years been bled. The remaining 79 per cent could have been justifiably considered as negative with reference to the whole blood test results. In the questionable group were included all animals that reacted in the 1:100 dilution in the tube test. All 14 of the animals which fell in this questionable group reacted in the whole blood test. The latter test also detected the 19 animals of the positive group; that is those that reacted in the 1:200 dilution in the tube test.

It might be pointed out in passing, that Table II also illustrates that the whole blood agglutination test, like the serum plate agglutination test detects residual antibody in a considerable proportion of vaccinated animals that would be reported as negative on the basis of results of tube agglutination tests.

As observed in Tables I and II, in a small number of the animals the degree of reaction in the whole blood test was not as strong as might have been anticipated in view of the high titer recorded in tube agglutination test. This point is exemplified further in Table III which lists 10 brucellosis-branded

**TABLE III**

*Comparison of Agglutination Tests with Serum and Whole Blood of Individual Brucellosis-branded Cattle*

<table>
<thead>
<tr>
<th>Herd Nos.</th>
<th>Cattle Nos.</th>
<th>Vaccination</th>
<th>Tube Test</th>
<th>Plate Test</th>
<th>Whole Blood Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dilution of Serum</td>
<td>Amount of Serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:25</td>
<td>1:50</td>
<td>1:100</td>
</tr>
<tr>
<td>13</td>
<td>17</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>&quot;</td>
<td>20</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&quot;</td>
<td>22</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&quot;</td>
<td>18</td>
<td>+</td>
<td>3</td>
<td>4</td>
<td>4</td>
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<tr>
<td>&quot;</td>
<td>23</td>
<td>—</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<tr>
<td>&quot;</td>
<td>53</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>&quot;</td>
<td>59</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>—</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* These reactions on serum and whole blood plate tests were very slow to appear and could easily have been overlooked if read too soon, before 10 to 15 minutes.
cattle some of which had contracted the infection many years previously. Some of these animals had apparently reached the stage of recovery. Three, Nos. 53, 59, and 14, gave atypical slow agglutination reactions both in the plate serum and the whole blood tests. Consequently, extra care should be taken when reading tests on animals that have contracted the infection many years earlier. Actually in official testing, “B” branded animals should not be retested.

In Table IV are given the reactions obtained with nine animals which had recently aborted. This table illustrates further that complete correlation is not always obtained between the clinical manifestations in the animal and the intensity of the agglutination reaction in the whole blood test.

**TABLE IV**

*Comparison of Agglutination Tests with Serum and Whole Blood of Individual Brucellosis Infected Cattle with a Recent History of Abortion*

<table>
<thead>
<tr>
<th>Herd Nos.</th>
<th>Cattle Nos.</th>
<th>Vaccination</th>
<th>Tube Test</th>
<th>Plate Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dilution of Serum</td>
<td>Amount of Serum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:25</td>
<td>1:50</td>
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<tr>
<td>1</td>
<td>4</td>
<td>+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>18</td>
<td>+</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>—</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>—</td>
<td>4</td>
<td>4</td>
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<tr>
<td>14</td>
<td>7</td>
<td>+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>—</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* This animal was with seven other animals that showed complete reactions to all tests.
CONCLUSION

The whole blood test performed with an hematoxylin-stained antigen to which anticoagulant in the form of heparin has been added, seems a promising tool to presumptively identify individual brucella-infected cattle. The sensitivity of the test as described is closely comparable to or a little more sensitive than the standard tube and plate serum agglutination tests. The sensitivity of the antigen can possibly be increased by changing the strain of *Br. abortus* or the culture-medium used for its cultivation.

However, with an increase in the sensitivity of the test, there will at the same time be an increase in the percentage of animals that react in the whole blood test but which will show only low-grade reactions in tube tests. Such reactions would in the final evaluation, be reported as negative.

In particular it is hoped that the whole blood test will prove of value in permitting a quick sorting out of the reactors from the normals among range cattle. The non-reacting individuals could be released from the corrals within less than half an hour.

The reactors could be retained, bled, and held in confinement until the results of the standard agglutination test are received. Such a program would also reduce the volume of testing in the diagnostic laboratories.

ACKNOWLEDGMENT

Appreciation is expressed to Dr. F. O. Read, Health of Animals Division, for cooperation in the field study. The authors also wish to thank Messrs. W. A. Boyd and G. Goyette for technical assistance in the experimental work.

REFERENCES


RESUME OF BRUCELLOSIS MEETINGS HELD IN LIMA, PERU

C. A. MANTHEI. D.V.M.*

This report is a résumé of three brucellosis meetings, two of which were Inter-American and the other international in scope. Although each meeting was held for a different purpose, all being concerned with the same disease makes it difficult to avoid some repetition or presentation of subject matter that is likely to be common knowledge to an audience such as the one assembled here. Few diseases have been investigated as extensively as brucellosis, but there is still need for additional information to adequately control this disease in all species of animals and under all kinds of economic and environmental conditions.

Training Course on Brucellosis

The first part of the résumé concerns the training course on brucellosis sponsored by the Pan American Sanitary Bureau. This was the third of a series of four such courses to be held in Latin America. The first two were for the purpose of instructing technical personnel in the production and standardization of diagnostic Brucella antigens, and in the techniques involved in their application. The fourth will be for the purpose of training technical personnel in production, standardization, and control of Strain 19 vaccine.

The third training course, which was held from September 30 through October 5, 1957, consisted of lectures and seminars on the research, control, and public health aspects of brucellosis. Fifty-seven medical doctors and veterinarians participated, 46 of whom were representatives from Latin American countries. The remaining 11, who served as lecturers or moderators, were representatives of Pan American Sanitary Bureau, Agricultural Research Service, and National Institutes of Health.

Discussions on research covered the broad fields of causative agents, diagnostic procedures, induced immunity and natural resistance, chemotherapy, and pathogenesis. Emphasis was given to accurate classification of isolations of Brucella and its importance in the study of the epidemiology of brucellosis. This involves application of standard biochemical and serological agents and procedures. Another topic that was thoroughly discussed and is closely related to epidemiology is the natural selective infectivity of the three classical species of Brucella for certain animal hosts, and the natural resistance or susceptibility of each host to the three species of Brucella. The evidence indicates that although cross infection occurs in most species of susceptible animals with the three species of Brucella, cattle are the principal host of Brucella abortus, swine of Brucella suis, and goats of Brucella meli-

* Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
tensis. The exceptions to this in some regions of the world are buffalo that harbor Br. abortus, a milking-type of sheep that harbor Br. melitensis, and wild hare that harbor Br. suis. When cross infection occurs in a non-specific host, the disease tends to be asymptomatic and self-limiting. Regardless of this, the significance of cross infection in the non-specific hosts should not be minimized because such animals may be a potential source of infection for the specific hosts.

Since the Latin Americans were acquainted with production and standardization of diagnostic antigens and standard techniques, discussion on diagnosis was limited mostly to proper use of antigens and interpretation of reactions. Although conditions in some countries prevent the fullest use of standard diagnostic biologics and methods; there is no lack of appreciation among technical personnel of the different countries for the need and advantages of having uniformity in diagnosis.

The stability, safety, immunogenicity, and control of Strain 19 were thoroughly reviewed because vaccination is the principal method of control of bovine brucellosis in many of the Latin American countries. This does not mean that a single approach to control is the one of choice, but it frequently has been the one of necessity because of existing conditions, such as shortage of technical personnel, high incidence of infection, inadequate financial support, etc. The things most emphasized were proper handling of vaccine to insure a potent product, vaccination by the recommended subcutaneous method and with the five-ml. dose to more nearly insure maximum protection, and vaccination of calves between four and eight months of age. The last point was emphasized and re-emphasized to impress upon them the importance of avoiding residual vaccinal titers in future programs involving use of the seroagglutination test for removal of reactors. Some of the control officials in this country have been confronted with the same problem when a brucellosis eradication program was initiated in areas where promiscuous vaccination had been practiced.

Considerable time was spent in discussing the degree and duration of protection induced with Strain 19 in cattle vaccinated as calves. Research has clearly demonstrated that immunity produced by Strain 19 in cattle may vary considerably between individuals, however it does not decrease as the age of the individual animal increases.

There was marked interest by the Latin Americans in the progress of the bovine brucellosis-eradication program in the United States. The broad subjects discussed were organization of national and local committees, determination of the economic and public health significance of the disease, development and design of a cooperative brucellosis program, necessity of uniform methods and rules, and progress of the brucellosis program in the United States. It was pointed out that the committees are usually organized along the same levels as political subdivisions, but regardless of how this is done they should consist of various segments of the livestock industry and disease control officials. Their purpose is to assemble and disseminate information to interested groups and the general public, organize support for the program,
and aid in developing a genuinely coordinated effort of cooperating groups. Determination of the economic and public health significance provides a source of knowledge which is essential in the development of any disease-control program. The design of the program must not only be technically sound but adaptable to existing conditions of a country. Success of a program is dependent to a large extent on employment of standard diagnostic products and procedures, proper use of a serviceable immunizing agent, disposal of infected animals, and promulgation and enforcement of uniform regulations for the entire activity. In addition to recommending what should be done, the mistakes made in this country were pointed out so that they may be avoided in the development of future programs in other countries.

Another subject matter discussed at the training course was the public health aspects of brucellosis. Every effort should be made to isolate Brucella from suspected cases of brucellosis and to classify accurately any isolations so that the source of exposure to infection may be determined. Since infected animals and their by-products are the principal sources of exposure to man, brucellosis of human populations can be prevented to a large extent by pasteurization of milk, thorough cooking of meat, application of sanitary measures, and control of brucellosis in animals. One of the most instructive parts of the discussion was clearly defining the procedures for making an accurate diagnosis of brucellosis of man, the various clinical manifestations of the disease, the value and limitation of chemotherapy, and the importance of supportive treatment of the patient. Accuracy of diagnosis, medical history, and clinical pattern of the disease are the factors that dictate the choice of method of treatment. As these preventive measures become routine, there will be a marked tendency for brucellosis to be limited to persons whose occupation places them in frequent contact with infected animals or their contaminated by-products.

Most members of the course participated freely in the discussion and presented results of studies in their country. Many presented data on the incidence and prevalence of brucellosis in human beings and animals, as well as information on methods employed in control of the disease. A very encouraging note was the enthusiasm and belief by veterinarians and the medical personnel of Latin America that the disease can be controlled regardless of the difficulties encountered in many of their countries. Some of these difficulties are shortage of communication facilities, limited financial support, and lack of understanding of the problem and consequently lack of interest. Regardless of these obstacles, technical personnel have initiated programs that provide partial control of the disease in many countries of Latin America. Vaccination with Strain 19 has been practiced in countries that have large cattle populations. Much of the vaccination has been done in adult cattle or sexually mature heifers, a procedure that interferes with a testing program. Swine brucellosis is considered a problem only in a few countries where the industry is well developed. Br. melitensis infection is prevalent in all countries that have a relatively large goat industry. Brucellosis in human beings, particularly that caused by Br. melitensis, is a serious problem in these
RESUME OF BRUCELLOSIS MEETINGS

The seriousness of the problem is directly related to the use of unpasteurized milk and milk products, as well as management practices which result in a close relationship between the owners and their goats.

Because of the relatively high incidence of human brucellosis, many Latin American countries have considered a bim ministerial approach to planning and directing brucellosis programs. My understanding of the procedure is that a brucellosis committee, made up of personnel from the ministries of agriculture and public health, would be responsible for and have the authority to conduct these programs. The operative mechanism of such a committee would no doubt vary among countries.

The progress of the brucellosis-eradication campaign in the United States is being watched very closely by our neighbors in South and Central America. The success that we attain will have a marked influence on future brucellosis-control programs in those countries. This should likewise cause us to consider fully our position on the control of brucellosis in species of animals other than cattle. Moreover, it appears to be another excellent opportunity to advance our philosophy that it is more economical to live without, than to live with, diseases.

Fourth Inter-American Congress on Brucellosis

The second part of the résumé concerns the Fourth Inter-American Congress on Brucellosis, held in Lima, Peru, October 6-8, 1957. Before entering into a discussion of the technical presentations, I will give a short history of the origin, development, and objectives of this organization.

The First Congress was held in Mexico in 1946 and was attended by persons from many countries of the Americas who were interested in brucellosis. It was at this time that they elected the Inter-American Committee on Brucellosis to provide the leadership for the group. The objectives of the organization were to promote research, to exchange information, and to develop a better understanding of brucellosis as it exists in the various countries. After the Second Congress, which was held in Buenos Aires, Argentina, in 1948, the Pan-American Sanitary Bureau has taken on the responsibility of lending support to the organization. A Third Congress was held in Washington, D.C., in 1950. Many of its members were invited to participate in the first meeting of the world-wide Expert Committee on Brucellosis following the Congress. During the Fourth Congress, an advisory committee was established. This committee is made up of a representative from each American country, who will aid the Inter-American Committee in planning future Congresses.

Approximately 100 persons attended the Fourth Congress, held in Lima, and all but three or four countries of the Americas were represented. There were approximately 35 papers presented, which covered practically all phases of the brucellosis problem. In addition, there were four panel discussions on the following subjects: principals of bovine brucellosis control, diagnosis of human brucellosis, brucellosis of goats, and public health aspects of brucellosis.
The papers relating to animals were on brucellosis of cattle and goats only. Those on cattle were concerned principally with epidemiological studies, methods of diagnosis, and methods of control in Latin American countries. An epidemiological survey in one country showed that *Br. melitensis* was occasionally isolated from cattle located in areas where a high incidence of *Br. melitensis* infection occurred in goats. A study on the fluctuation of titers of approximately 100 vaccinated cattle at the time of parturition showed that a high percentage of animals had a decrease in titers at the time of parturition. These titers usually increased to the preparturition level within about two weeks after parturition. This subject was of considerable interest because sporadic reports in this country indicate that titers increase at the time of parturition. A limited review of our work indicates there is little or no relationship between fluctuation of vaccinal titers and parturition. These differences indicate a need for further study to clarify the situation. Interference of persistent titers in adult vaccinated animals with the seroagglutination test is a problem in some of the countries. There is considerable interest in differentiating of vaccinal and infectional titers. This situation is not an unfamiliar one in this country, and the most promising way of avoiding it is to vaccinate heifer calves at the recommended age. The surface-fixation test was compared with the seroagglutination test for the diagnosis of brucellosis in cattle. It was the opinion of the authors that the reactions obtained with the surface-fixation test were specific for brucellosis. Since the test did not give qualitative results, more studies must be conducted before its diagnostic significance can be ascertained.

There was also much interest in the papers on the natural course of *Br. melitensis* infection in goats, vaccination of goats against *Br. melitensis* infection, and methods of diagnosing *Br. melitensis* infection in goats. The natural course of *Br. melitensis* of goats appears to follow a pattern similar to that of *Br. suis* infection in swine. The rate of recovery of infected goats and swine is relatively high and is associated with a rapid decline in titers; consequently some animals harbor infection when showing a titer below the 1:100 level. This presents a problem of having a reservoir of infection that may be undiagnosed with the standard seroagglutination tests. There was also considerable interest in studies on vaccination of goats against *Br. melitensis* infection with a vaccine prepared from a streptomycin non-dependent strain of *Br. melitensis*. Results presented at the Congress indicate more research is required to prove that the virulence of this strain is stable and is reduced to the degree where it will not produce an infection which spreads by contact from inoculated pregnant goats to susceptible pregnant goats.

A number of interesting papers were presented on brucellosis of human beings and public health aspects of the disease. Epidemiological studies in Latin America show that *Br. melitensis* is the leading cause of brucellosis of man in some countries. Another report was on differences in the incidence of infection in workers in different abattoirs. This difference in incidence of infection appeared to be related to the difference in type and age of cattle slaughtered in the respective establishments. A very encouraging note was
the general agreement of the medical people on the methods of diagnosing brucellosis of human beings and on the role of chemotherapy in treating this disease. The reasons for this better agreement are greater general use of standard diagnostic agents and techniques and a better understanding of each other's points of view which is the result of thorough discussions and of minimizing their differences on minor points.

It is planned to publish all papers presented at the Congress in a Proceedings Book. In addition, permission was granted to authors to publish individual papers in technical journals to obtain wider distribution of the information.

Third Meeting of the Joint FAO/WHO* Expert Committee on Brucellosis

The last part of the résumé concerns the deliberations and recommendations of the Joint FAO/WHO Expert Committee on Brucellosis at the meeting held October 9-15, 1957, in Lima, Peru. This was the third meeting of the Committee, the first two of which were held in Washington, D.C., and Florence, Italy, in 1950 and 1952, respectively. The Expert Committee on Brucellosis was initiated in 1950, and its members were selected on the basis of background of specific subject matter and due regard to geographical distribution. Reports of the Committee express the corporate views of the members and are of basic importance as guides to the FAO/WHO organizations in the development of policies and programs.

The members selected to attend the third meeting of the Brucellosis Committee in Lima reviewed progress of programs and results of research since 1952. After these items were duly considered, the information believed to be of value in control of brucellosis in the future was incorporated in the report. Also included in the report were recommendations for further research in areas of the brucellosis problem where more information is needed. Copies of this, as well as the first two reports, can be obtained from International Documents Service, Columbia University Press, 2960 Broadway, New York 27, New York.

Although practically all phases of brucellosis were studied, those that received special attention were pathogenesis of \textit{Br. melitensis} infection of goats, vaccines for the protection of goats against \textit{Br. melitensis}, methods of diagnosing brucellosis of goats, vaccination of human beings against brucellosis, addition of new species to the genus \textit{Brucella}, vaccines and vaccination of cattle, and prevalence of swine brucellosis.

Since the first three items come under the general heading of caprine brucellosis, they will be discussed together. Abortions are not necessarily the principal symptoms of caprine brucellosis. Because of the tendency toward a relatively high rate of recovery within one to two years after infection becomes established, there is a gradual recession of seroagglutinin titers during this period. It is at the terminal part of the recovery period or of varying periods thereafter that some goats continue to shed \textit{Br. melitensis} in

* Food and Agriculture Organization/World Health Organization.
the absence of diagnostic titers. This creates a problem of making an accurate diagnosis with the seroagglutination methods. Of the tests studied, a modified tube agglutination, flocculation, and Comb's tests appeared to give a more accurate appraisal of Br. melitensis infection of goats than the standard seroagglutination procedures. A number of vaccines have been studied to determine their immunizing qualities against Br. melitensis of goats. The three that showed the most promise were a heat-killed smooth Br. melitensis suspended in an adjuvant, a heat-killed rough Br. melitensis suspended in an adjuvant, and a living vaccine prepared from a streptomycin non-dependent strain of Br. melitensis which had been selected from the population of a streptomycin dependent strain. After evaluation of the immunogenic and safety qualities, the killed smooth Br. melitensis vaccine was the one selected for critical field trials. Additional research was recommended to determine more critically the degree and stability of the virulence of the streptomycin non-dependent strain of Br. melitensis before a living vaccine prepared from it could be considered for similar field trials.

The use of living Brucella vaccines for the prevention of brucellosis of human beings was introduced for discussion because of the claims made for two such vaccines by the Russians. One of these vaccines was prepared from colonies selected from a culture of Strain 19. Absence of detailed information on the experiments conducted in Russia, knowledge of proved cases of accidental infection of human beings with Strain 19 in the United States, and the inability of Strain 19 to provide serviceable protection in swine against Br. suis and in goats against Br. melitensis resulted in a recommendation of the Committee against use of a living Brucella vaccine in people.

The subject of adding new species to the genus Brucella always stimulates active discussions. The two microorganisms proposed as new species were (1) a bacterial agent that causes infertility and a low incidence of abortion in sheep and which was named Brucella ovis by its discoverers, and (2) a strain of Brucella that has biochemical characteristics of Br. melitensis and serological characteristics of Br. abortus. The name suggested for the latter was Brucella intermedia. Because of the disagreement among the group on acceptance of the proposal and on what criteria are most significant in designating new species of Brucella, the entire question was referred to the International Committee on Bacterial Nomenclature for study. The Expert Committee on Brucellosis also recommended that isolation of aberrant Brucella in the future should be widely distributed among investigators for detailed study before designating any of them as new species of the genus Brucella.

Since Strain 19 vaccination is an integral part of bovine brucellosis-control programs in many countries throughout the world, definite recommendations were made on the handling and use of the vaccine to insure maximum results. Data which have been available for some time show that freeze-dried vaccine reconstituted to the liquid form loses its viability within a few days at temperatures of 27°C to 37°C. Recent data also show that vaccine in the dried form maintains its viability for at least one year when stored at temperatures of 4°C-7°C, but loses its viability rapidly within a few weeks
when kept at 27°C. and more rapidly at 37°C. Consequently, the necessity for holding all types of Strain 19 vaccines at 4°C-7°C. to insure maximum viability at the time they are used cannot be overemphasized. The results of three large controlled laboratory experiments (two at Beltsville, Maryland, and one at Compton, England) and two large field experiments (one in the United States and the other in New Zealand) show that immunity induced in calf-vaccinated cattle with Strain 19 does not decrease as the animals become older. This is reflected in the failure to increase significantly the immunity in calf-vaccinated animals by revaccination. Since all the animals involved in these studies received the five-ml. dose (approximately 50 x 10⁹ viable cells) of Strain 19 vaccine by the subcutaneous method and significant losses in viability are known to occur in vaccines that are improperly handled, it was emphatically recommended that Strain 19 vaccine be administered in the five-ml. dose or its equivalent in viable cells by the subcutaneous method until such time that a more stable product is developed and adequate data available to justify use of a smaller vaccinal dose.

A number of reports indicate that swine brucellosis has become more prevalent in some countries and is considered a major disease.

The seroagglutination test is still considered to be the most reliable diagnostic method for detection of brucellosis in the individual bovine animal.

The final action of the Committee was a recommendation on future research. Some of the general suggestions for study were pathogenesis of brucellosis in animals, particularly sheep, immunization of goats against infection with Br. melitensis, standardization of reagents and techniques of diagnostic tests, non-specific reactions in sera and milk, development and mechanism of immunity, further adoption of screening tests for locating infection in various species of animals, and development or improvement of tests for the diagnosis of brucellosis of swine, goats, and sheep.
COOPERATIVE STATE-FEDERAL BRUCELLOSIS ERADICATION


The optimism reflected in our report to this Association last year has been fully justified by progress made in the cooperative State-Federal bovine brucellosis eradication campaign for the twelve-month period ending June 30, 1957. Although certain factors continue to retard the program to some extent they are becoming much less important than ever before.

It is most encouraging to note the continued strong support being given the eradication project by the livestock industry. This has resulted in some difficulties being experienced in meeting service requirements of the program. There appears to be a strong determination on the part of all interested groups to once and for all eliminate the long-time economic and public health threat from brucellosis.

The effectiveness of existing tools and procedures continues to be demonstrated in wide-scale use throughout the country. This does not mean, of course, that further improvements are impossible. No doubt refinements of old and the development of some new techniques will be needed in the final phases of the eradication effort. In fact, studies already are being carried out that should provide for more rapid progress toward final eradication in areas where the incidence of infection has been reduced to a very low level.

First 20 Years of the Cooperative Bovine Brucellosis Eradication Campaign—1934-1954

During the period of 1934 through 1954 there were nearly 11 million herds representing approximately 127 million cattle blood-tested for brucellosis. Over the same period, herd and animal infection rates were reduced from about 38 percent to 14.2 percent and from nearly 10 percent to 2.6 percent respectively.

While Strain 19 vaccine was not approved until 1941, there were nearly 20 million calves vaccinated during the following 13 years. The use of vaccine has increased each year since it was accepted as part of the official program. While it is impossible to accurately evaluate the benefits derived from vaccination, there is increasing evidence that it has played an important part in reducing the previously high incidence of infection in certain areas. The need for providing a serviceable degree of protection in otherwise susceptible cattle will continue until complete eradication of bovine brucellosis is achieved.

Although this phase of the program was plagued by numerous interfering factors a great deal was accomplished and even more learned about com-

1 Dr. C. K. Mingle, Chief, Brucellosis Eradication Section, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
batting brucellosis from actual experience. By recognizing these early mistakes it has been possible to avoid repetition, thereby improving the effectiveness of the procedures employed in the program.

First Three Years of the Accelerated Bovine Brucellosis Eradication Campaign—1955-1957

When additional Federal funds for the eradication of bovine brucellosis first became available in the early part of fiscal year 1955, immediate steps were taken to expand the program on a nation-wide basis. The rapidity with which it was possible to accelerate bovine brucellosis eradication activities indicates the high interest in the program that already existed.

Tables I and II show the increased level of program operations that have been attained during the first three years of the stepped-up project. It will be noted that all phases of the program were greatly increased over previous levels.

Blood Testing

Over this three-year period there was a total of 46.8 million cattle in 3.3 million herds blood-tested for brucellosis. Of this number, 2.2 percent of the cattle and 12.8 percent of the herds showed evidence of infection. It will be noted in Table I that in comparison with the preceding three-year period, this represents an increase of 63.3 percent in the number of herds tested, 98.4 percent increase in cattle tested and a reduction of 3 percent and 1.2 percent in respective infection rates.

Brucellosis Ring Testing

Even though use of the BRT has increased each year during the expanded program, it is significant that these increases have been associated with reduced percentages of ring-suspicious herds. For fiscal years 1955 through 1957 there were 4.7 million herds ring tested, of which 15.6 percent was classed as suspicious. These figures represent an increase of 133 percent in the number of herds tested and a reduction of 11.4 percent in the number of suspicious herds found as compared with the preceding three-year period.

In spite of certain recognized limitations, the BRT is providing an excellent means of detecting new infection foci in dairy areas before they are able to cause serious extension of the disease. Whereas area certifications in dairy sections have been difficult to maintain before the ring-test was available, this problem is no longer one of serious consequence when the BRT is properly applied. This procedure is now being used to some extent in all of the States and Puerto Rico. Its economy of application and practicability highly recommends the widest use possible of the ring test.

Vaccination

Continued increases have been noted in all three years of the accelerated program. This continues an unbroken trend beginning at the time vaccine
## TABLE I

### Comparative Brucellosis Data

<table>
<thead>
<tr>
<th>Activities</th>
<th>1952</th>
<th>1953</th>
<th>1954</th>
<th>Total</th>
<th>1955</th>
<th>1956</th>
<th>1957</th>
<th>Total</th>
<th>Per Cent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds Tested</td>
<td>670,738</td>
<td>660,344</td>
<td>696,207</td>
<td>2,027,289</td>
<td>994,541</td>
<td>1,154,962</td>
<td>1,170,906</td>
<td>3,310,409</td>
<td>63.3+</td>
</tr>
<tr>
<td>Reactor Herds</td>
<td>115,687</td>
<td>108,085</td>
<td>96,238</td>
<td>320,010</td>
<td>143,690</td>
<td>156,406</td>
<td>123,964</td>
<td>424,060</td>
<td>32.5+</td>
</tr>
<tr>
<td>Per Cent</td>
<td>17.2</td>
<td>16.4</td>
<td>13.8</td>
<td>15.8</td>
<td>14.6</td>
<td>13.5</td>
<td>10.6</td>
<td>12.8</td>
<td>(3.0—)</td>
</tr>
<tr>
<td>Cattle Tested</td>
<td>7,491,327</td>
<td>7,860,870</td>
<td>9,002,109</td>
<td>24,354,306</td>
<td>14,186,241</td>
<td>16,754,195</td>
<td>15,913,396</td>
<td>46,853,832</td>
<td>92.4+</td>
</tr>
<tr>
<td>Reactor Cattle</td>
<td>314,260</td>
<td>268,348</td>
<td>235,666</td>
<td>818,274</td>
<td>365,247</td>
<td>366,524</td>
<td>280,253</td>
<td>1,012,024</td>
<td>23.7+</td>
</tr>
<tr>
<td>Per Cent</td>
<td>4.2</td>
<td>3.4</td>
<td>2.6</td>
<td>3.4</td>
<td>2.6</td>
<td>2.2</td>
<td>1.8</td>
<td>2.2</td>
<td>(1.2—)</td>
</tr>
<tr>
<td>Reactors Slaughtered</td>
<td>95,600</td>
<td>85,836</td>
<td>120,877</td>
<td>362,313</td>
<td>259,781</td>
<td>327,034</td>
<td>266,594</td>
<td>853,409</td>
<td>182.3+</td>
</tr>
<tr>
<td>Per Cent</td>
<td>31.4</td>
<td>32.0</td>
<td>51.3</td>
<td>36.9</td>
<td>71.1</td>
<td>89.2</td>
<td>95.1</td>
<td>84.3</td>
<td>(47.4+)</td>
</tr>
<tr>
<td>Ring Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds Tested</td>
<td>454,732</td>
<td>670,532</td>
<td>932,003</td>
<td>2,057,267</td>
<td>1,200,898</td>
<td>1,727,531</td>
<td>1,866,444</td>
<td>4,794,923</td>
<td>133.1+</td>
</tr>
<tr>
<td>Susp. Herds</td>
<td>135,967</td>
<td>175,909</td>
<td>242,914</td>
<td>554,790</td>
<td>278,847</td>
<td>255,503</td>
<td>212,580</td>
<td>746,930</td>
<td>34.6+</td>
</tr>
<tr>
<td>Per Cent</td>
<td>30.0</td>
<td>26.2</td>
<td>26.1</td>
<td>27.0</td>
<td>23.2</td>
<td>14.8</td>
<td>11.4</td>
<td>15.6</td>
<td>(11.4—)</td>
</tr>
<tr>
<td>Vaccinations</td>
<td>3,179,251</td>
<td>3,688,149</td>
<td>3,999,101</td>
<td>10,866,501</td>
<td>4,381,397</td>
<td>4,772,335</td>
<td>5,501,445</td>
<td>14,655,377</td>
<td>34.9+</td>
</tr>
<tr>
<td>Certifications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Counties</td>
<td>18</td>
<td>46</td>
<td>33</td>
<td>97</td>
<td>46</td>
<td>169</td>
<td>241</td>
<td>456</td>
<td>370.1+</td>
</tr>
<tr>
<td>Counties Removed</td>
<td>98</td>
<td>79</td>
<td>11</td>
<td>183</td>
<td>1</td>
<td>48</td>
<td>6</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Total Cert. Counties</td>
<td>345</td>
<td>312</td>
<td>334</td>
<td>334</td>
<td>379</td>
<td>500</td>
<td>735</td>
<td>735</td>
<td>120.1+</td>
</tr>
</tbody>
</table>

( ) Per cent difference.
TABLE II
Comparative Brucellosis Data

<table>
<thead>
<tr>
<th>Activities</th>
<th>1956</th>
<th>1957</th>
<th>Per Cent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood Tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Herds Tested</td>
<td>1,154,962</td>
<td>1,170,906</td>
<td>1.38+</td>
</tr>
<tr>
<td>No. Reactor Herds</td>
<td>156,406</td>
<td>123,964</td>
<td>26.17—</td>
</tr>
<tr>
<td>Per Cent</td>
<td>13.5</td>
<td>10.6</td>
<td>(2.9—)</td>
</tr>
<tr>
<td>No. Cattle Tested</td>
<td>16,754,195</td>
<td>15,913,396</td>
<td>5.02—</td>
</tr>
<tr>
<td>No. Reactor Cattle</td>
<td>366,524</td>
<td>280,253</td>
<td>23.54—</td>
</tr>
<tr>
<td>Per Cent</td>
<td>2.19</td>
<td>1.76</td>
<td>(.43—)</td>
</tr>
<tr>
<td>Reactors Slaughtered</td>
<td>327,034</td>
<td>266,594</td>
<td>18.48—</td>
</tr>
<tr>
<td>Per Cent</td>
<td>89.23</td>
<td>95.13</td>
<td>(5.90+)</td>
</tr>
<tr>
<td><strong>Ring Tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Herds Tested</td>
<td>1,727,581</td>
<td>1,866,444</td>
<td>8.04+</td>
</tr>
<tr>
<td>No. Susp. Herds</td>
<td>255,503</td>
<td>212,580</td>
<td>16.80—</td>
</tr>
<tr>
<td>Per Cent</td>
<td>14.7</td>
<td>11.4</td>
<td>(3.4—)</td>
</tr>
<tr>
<td><strong>Vaccinations</strong></td>
<td>4,772,535</td>
<td>5,501,445</td>
<td>15.27+</td>
</tr>
<tr>
<td><strong>Certifications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Counties</td>
<td>169</td>
<td>241</td>
<td>42.6+</td>
</tr>
<tr>
<td><strong>Counties Removed</strong></td>
<td>48</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total Cert. Counties</td>
<td>500</td>
<td>735</td>
<td>47.0+</td>
</tr>
</tbody>
</table>

( ) Per cent difference.

was first approved for official use in 1941. During fiscal years 1955, 1956 and 1957 a total of 14.6 million official vaccinations were reported. This represents an increase of 34.9 percent over the 10.8 million recorded for the preceding three-year period.

There is no question about the help Strain 19 vaccine has provided in reducing the incidence of infection in many cases to a level that made actual eradication practices economically possible. Numerous sections of the country which were originally heavily infected are now experiencing very little difficulty in qualifying for certification, due largely to extensive use of vaccine. With vaccination being employed more and more as an adjunct to test and elimination of reactors, this procedure is making the eradication of brucellosis more easily accomplished. The poor judgment previously observed in the use of vaccine has now become the exception rather than the rule.

**Area Certification**

Although it is possible to carry out brucellosis eradication on an individual herd basis, difficulties are often encountered in keeping such herds free of the disease, especially when they are located in regions where considerable infection exists. For this and other reasons, every effort is made to encourage complete area work in the accelerated campaign.
During the first three years of the expanded program, 456 new county certifications were recorded for the country as a whole. This compares with 97 initial certifications made during the previous three-year period. Moreover, during this latter period 86 certifications were lost due to failure of counties to qualify on a recertification test.

As of June 30, 1957, there were 44 states in which area work was being carried out in a total of 1,447 counties. Of these 1,447 area counties, 735 including seven complete states were listed as certified. The remaining 712 were operating programs designed to achieve early recognition as certified areas. Thus, out of a total of 3,150 counties in the United States, Puerto Rico and the Virgin Islands, 46 percent were either currently certified or rapidly nearing that status on June 30, 1957.

Future Outlook

The States of Vermont and Connecticut together with 66 more counties have been added to the certified list during the first three months of the current fiscal year. This makes a total of 823 counties including nine complete states qualified for certification as of September 30, 1957. It is anticipated that at least 800 additional counties including seven more states will certify during the present fiscal year. This would give us a total of 16 states and about 50 percent of all counties being certified as of June 30, 1958. It also is expected that by then most, if not all, states will be conducting complete area programs.

In comparing activities for fiscal years 1956 and 1957, Table II, it would appear that brucellosis program operations should continue at about the same level during the current fiscal year. From that point on it is reasonable to expect a gradual decline in the volume of field activities. It will be noted that activities for 1956 and 1957 were comparable in most respects.

While many states have set 1960 as the goal for certification, this will not be possible in all cases. However, if advantage can be taken of the present momentum through continued adequate financial support, the entire country should be certified within the next five years.

It is important, of course, to recognize the fact that certification alone does not represent actual eradication. Until such time as brucellosis is completely eliminated from all susceptible species of livestock, our efforts must be continued. There is reason to believe that final eradication can be achieved if we so desire.

Meeting the necessary manpower requirements has been the most difficult problem encountered so far in the accelerated program. Although nearly 6,000 accredited practitioners are listed for fee-basis work, only about 60 percent of them are ever working at the same time. Therefore, in many instances, this limited help is inadequate to meet service requirements. A recent survey indicated that failure of fee-basis veterinarians to adequately participate in the program was the primary reason for delayed progress in 15 states. Success of this project is largely dependent upon the active cooperation and support of practicing veterinarians.
SUMMARY

1. Extensive field experience has shown that intelligent use of the standardized blood serum agglutination test, the brucellosis ring test, and Strain 19 vaccine can be used effectively to control and eradicate bovine brucellosis.

2. During the three-year period ending June 30, 1957, a total of 46.8 million cattle were blood tested for brucellosis in 3.3 million herds. Of these, 2.2 percent of the cattle and 12.8 percent of the herds showed evidence of infection. These figures compare with 3.4 percent animal and 15.8 percent herd infection rates disclosed during the preceding three-year period.

   For fiscal year 1957 official blood tests were conducted on 15.9 million cattle in 1.1 million herds. Results from these tests indicated an infection rate of 1.8 percent cattle and 10.6 percent herd.

3. The establishment and maintenance of Modified Certified Brucellosis-Free Areas is progressing in a satisfactory manner. As of September 30, 1957, there were 823 certified counties, including nine complete states and 706 other counties actively working toward early certification.

4. Even though area certifications are fundamental to the program, sight must not be lost of the ultimate goal—eradication. The benefits to be derived from this achievement will more than repay the continued effort required.

5. The cooperative State-Federal bovine brucellosis eradication campaign has never enjoyed stronger support from all interested groups. By maintaining the momentum generated over the past few years through adequate State and Federal financing, the incidence of this disease should be reduced to 1 percent or less within the next five years.

6. Brucellosis can be eradicated.
A SUMMARY OF THE STUDIES IN MINNESOTA ON THE WHEY PLATE TEST FOR THE DIAGNOSIS OF BOVINE BRUCELLOSIS*

M. H. ROEPKE, Ph.D.**, F. C. STILES, Jr., D.V.M.***, and
F. C. DRIVER, D.V.M.***, St. Paul, Minnesota

This report is a summary of two different studies (1, 2) comparing the whey test described by Cameron and co-workers (3-6) with the standard seroagglutination test for the diagnosis of bovine brucellosis under the conditions prevailing in Minnesota.

In conducting the whey plate tests for the two studies the incubation period used for the test was six minutes, as recommended by Cameron, Kendrick, and Merriman (4). The data reported in the first study (1) were given in terms of a six-minute incubation period. After the completion of the second study a report by Cameron (6) indicated that the incubation period used for the whey plate test was 10 minutes. Fortunately, the whey test reactions in the two Minnesota studies were read after both six and 14 minutes incubation. This was done to compare the sensitivity of the whey plate test with the milk plate test. The report by Blake, Manthei and Goode (7) indicated that a 14-minute incubation period provided for a maximum sensitivity of the milk plate test. The same is true for the whey plate test (8).

As there was considerable uncertainty regarding a specific incubation period for the whey plate test (3-6), and since the first and second studies conducted in Minnesota were presented (1, 2) in terms of different incubation periods of the test (six and 14 minutes respectively), it seemed desirable to summarize the two studies in terms of a uniform incubation period of 14 minutes, which results in maximum reactions with the whey test.

MATERIALS AND METHODS

To a major extent the blood reactor animals involved in this study were found as a result of routine field retests of reactor and suspect herds and blood tests on ring test suspicious herds.

The major difference between the two studies is that the first study involved recently branded reactor (204) animals shipped to the South St. Paul Stockyards for slaughter and the second study concerned both blood and whey plate tests on all (1,289) milk producing animals in 51 blood test reactor herds and in 22 ring test suspicious herds that were either negative or suspicious to the blood test.

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*** Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, St. Paul.

The studies were supported in part by the Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture.

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One of the purposes of the second study was to obtain information of the relative frequency of blood reactor-whey negative animals as compared with blood negative-whey positive animals. Also, various tissues in addition to milk from eight animals were cultured to obtain some information on the reliability of culturing a single milk sample from an animal for the determination of infection.

The detailed information regarding the collection of the blood and milk samples, the techniques of conducting the various agglutination tests, and the methods of culturing milk and tissues are given in the two previous reports (1, 2) and will not be repeated here.

Except where specifically noted, the results of the milk and whey plate tests are given in terms of a 14-minute incubation period for both tests. The reactions were recorded as any agglutination in the least amount of milk or whey, i.e., 0.08-ml milk or whey = 1+, 0.04-ml = 2+, 0.02-ml = 3+, 0.01-ml = 4+, and 0.005-ml = 5+. The recommendations of Cameron (5) were followed in the interpretation of the whey plate test reactions, namely, a negative or a 1+ reaction as negative, a 2+ reaction as suspicious, and a 3+ or higher reaction as positive.

RESULTS

Negative whey tests were obtained on 41 (20 percent) of the 204 reactor animals in the first study and on 21 (24 percent) of the 88 reactor animals in the second study.

A comparison of the blood and whey plate tests on the total of 292 blood test reactor animals is summarized in Table I.

<table>
<thead>
<tr>
<th>Blood Test</th>
<th>Whey Plate Test—No. Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer*</td>
<td>No. of Animals</td>
</tr>
<tr>
<td>+ 100</td>
<td>65</td>
</tr>
<tr>
<td>100</td>
<td>32</td>
</tr>
<tr>
<td>+ 200</td>
<td>39</td>
</tr>
<tr>
<td>200</td>
<td>17</td>
</tr>
<tr>
<td>400</td>
<td>59</td>
</tr>
<tr>
<td>800</td>
<td>44</td>
</tr>
<tr>
<td>1,600</td>
<td>29</td>
</tr>
<tr>
<td>3,200</td>
<td>3</td>
</tr>
<tr>
<td>4,000</td>
<td>3</td>
</tr>
<tr>
<td>12,800</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
</tr>
<tr>
<td>Per Cent of Total</td>
<td></td>
</tr>
</tbody>
</table>

* Titters of + 100 through + 200 are plate test titers, and the remainder are tube test titers.
Negative whey tests were obtained on 62 (21 percent) of the 292 blood reactor animals. Ninety-two percent (57 of 62) of the negative whey tests were on blood reactor animals with titers of +100 to +200 inclusive.

Of the 292 blood reactor animals, 25 (8.6 percent) were listed on the blood test charts as calf-vaccinated animals. Negative whey tests were obtained on seven (28 percent) and suspicious whey reactions on two (8 percent) of the blood test reactor vaccinated animals. No Brucella were isolated from the nine whey test negative or suspicious vaccinated animals. Brucella were isolated from 12 (75 percent) of the 16 blood-reactor-whey positive calf-vaccinated animals. The percent negative whey tests and the percent isolations of Brucella from the whey test positive calf-vaccinated blood reactor animals were not markedly different from the comparable values for non-vaccinated animals. Therefore, the calf-vaccinated reactor animals were included with the non-vaccinated animals in the over-all summary of the Brucella isolation studies shown in Table II.

### TABLE II

**Brucella Isolation Studies on 185 Blood Test Reactor Animals**

<table>
<thead>
<tr>
<th>Whey Plate Test</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg. 1+</td>
<td>2+</td>
<td>3+ 4+ 5+</td>
</tr>
<tr>
<td>No. Animals Cultured</td>
<td>53 9 22</td>
<td>30 18 53</td>
<td></td>
</tr>
<tr>
<td>No. Brucella Isolations</td>
<td>12 3 7</td>
<td>18 12 43</td>
<td></td>
</tr>
<tr>
<td>Per Cent Isolations</td>
<td>23% 33% 32%</td>
<td>60% 67% 81%</td>
<td></td>
</tr>
<tr>
<td>% Isol. Whey Neg.</td>
<td>24%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Isol. Whey Susp.</td>
<td></td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>% Isol. Whey Pos.</td>
<td></td>
<td></td>
<td>72%</td>
</tr>
</tbody>
</table>

Brucella were isolated from seven (17 percent) of the 41 blood reactor-whey negative animals in the first study and from eight (38 percent) of the 21 blood reactor-whey negative animals in the second study. This is a total of 15 (24 percent) Brucella isolations made from the 62 whey test negative animals with reactor blood test titers in the two studies as shown in Table II.

Brucella were isolated from seven (32 percent) of 22 blood reactor animals cultured that were whey test suspicious and from 73 (72 per cent) of the 101 blood reactor animals cultured that were whey test positive.

For the purpose of comparison, the results of the Brucella isolation studies were tabulated on the basis of a six-minute incubation period for the whey test. The data are presented in Table III.
SUMMARY FOR THE DIAGNOSIS OF BOVINE BRUCELLOSIS 121

TABLE III
Brucella Isolation Studies on 185 Blood Test Reactor Animals
(6-Minute Incubation)

<table>
<thead>
<tr>
<th>Whey Plate Test (6-minute incubation)</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg.</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>No. Animals Cultured</td>
<td>67</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>No. Brucella Isolations</td>
<td>16</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Per Cent Isolations</td>
<td>24%</td>
<td>37%</td>
<td>54%</td>
</tr>
<tr>
<td>% Isol. Whey Neg.</td>
<td>26%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Isol. Whey Susp.</td>
<td></td>
<td></td>
<td>54%</td>
</tr>
<tr>
<td>% Isol. Whey Pos.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The percent Brucella isolations in the blood reactor-whey negative group was essentially the same for the 14-minute incubation period (24 percent) as for the six-minute incubation period (26 percent). The chief difference was a decrease in the number of blood reactor-whey negative animals from 83 with a six-minute incubation period to 62 with a 14-minute incubation period for the whey test.

The 14-minute incubation period for the whey test resulted in the following changes in the tabulation of the results on the 67 animals with negative whey reactions found with the six-minute incubation period: 53 remained the same (12 Brucella isolations), seven changed to 1+ reactions (two Brucella isolations), six changed to 2+ reactions (two Brucella isolations) and one changed to a 3+ reaction (no Brucella isolated). The change for the 16 animals with 1+ whey reactions (six-minute incubation) were as follows: two remained the same (one Brucella isolation), 12 changed to 2+ reactions (four Brucella isolations) and two changed to 3+ reactions (one Brucella isolation). For the 22 animals with 2+ whey reactions (six-minute incubation) the changes were: four remained the same (two Brucella isolations), 17 changed to 3+ reactions (10 Brucella isolations) and one changed to a 4+ reaction (no Brucella isolated).

The results of various agglutination tests on the serum, milk and whey of the 15 blood reactor-whey negative animals from which Brucella were isolated are given in Tables IV and V. The distribution pattern of serum titers for these 15 animals was six (40 percent) 1+100, four (27 percent) 1 200, four (27 percent) 1+200, and one (7 percent) 1+400.


**TABLE IV**  
*Agglutination Tests on Seven Blood Reactor-Whey Negative Animals From Which Brucella Were Isolated (First Study)*

<table>
<thead>
<tr>
<th>An. No.</th>
<th>Serum Titer</th>
<th>Whey Plate Test</th>
<th>Ring Test Titer*</th>
<th>Milk Plate Test Quarter Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>+200</td>
<td>—</td>
<td>8</td>
<td>1+ — 1+ — — 2+</td>
</tr>
<tr>
<td>36</td>
<td>1 200</td>
<td>—</td>
<td>8</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>68</td>
<td>1 200</td>
<td>—</td>
<td>25</td>
<td>— — — — 3+ —</td>
</tr>
<tr>
<td>81</td>
<td>+200</td>
<td>1+</td>
<td>8</td>
<td>2+ 1+ 1+ —</td>
</tr>
<tr>
<td>96</td>
<td>+200</td>
<td>—</td>
<td>2</td>
<td>— — — — —</td>
</tr>
<tr>
<td>157</td>
<td>+200</td>
<td>—</td>
<td>8</td>
<td>— — — — —</td>
</tr>
<tr>
<td>162</td>
<td>1 200</td>
<td>1+</td>
<td>8</td>
<td>— 1+ 1+ — —</td>
</tr>
</tbody>
</table>

* Highest dilution in mixed negative milk at which a 2+ or stronger reaction was obtained.

**TABLE V**  
*Agglutination Tests on Eight Blood Reactor-Whey Negative Animals From Which Brucella Were Isolated (Second Study)*

<table>
<thead>
<tr>
<th>An. No.</th>
<th>Serum Titer</th>
<th>Whey Plate Test</th>
<th>Ring Test Titer**</th>
<th>Milk Plate Test Quarter Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+100</td>
<td>—</td>
<td>—1</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>6</td>
<td>1 200</td>
<td>—</td>
<td>8</td>
<td>2+ 2+ 1+ 2+</td>
</tr>
<tr>
<td>7</td>
<td>+100</td>
<td>—</td>
<td>25</td>
<td>2+ — — — —</td>
</tr>
<tr>
<td>9</td>
<td>+400</td>
<td>—*</td>
<td>8</td>
<td>— — — — —</td>
</tr>
<tr>
<td>11</td>
<td>+100</td>
<td>—</td>
<td>8</td>
<td>1+ 1+ 1+ —</td>
</tr>
<tr>
<td>68</td>
<td>+100</td>
<td>—*</td>
<td>—1</td>
<td>— — — — —</td>
</tr>
<tr>
<td>98</td>
<td>+100</td>
<td>—*</td>
<td>2</td>
<td>— 1+ — — 1+</td>
</tr>
<tr>
<td>108</td>
<td>+100</td>
<td>1+</td>
<td>12</td>
<td>— 4+ — — —</td>
</tr>
</tbody>
</table>

* Brucella isolated from tissues but not milk.

** Highest dilution in mixed negative milk at which a 2+ or stronger reaction was obtained, —1 = no reaction in undiluted milk.

As mentioned earlier, various tissues for culturing were obtained from eight blood test reactor animals that were negative to the whey test. *Brucella* were isolated from the tissues but not the milk of three of the eight animals. These three animals are designated in Table V. As may be noted in Table V, the milk plate reactions on the quarter milk samples from the three animals were negative for 10 quarter samples and 1+ for two samples. The culturing of the tissues as well as the milk from eight of the 21 blood reactor-
whey negative animals resulted in a 60 percent increase in the number of animals from which *Brucella* were isolated (an increase from five to eight).

To obtain information on the relative frequencies of blood reactor-whey negative animals as compared with blood negative-whey positive animals, blood and whey plate tests were made on all (1,289) lactating cows in 51 blood test reactor herds and in 22 ring test suspicious herds classified as negative or suspicious by the blood test. The tests are summarized in Table VI. There were 21 instances of blood reactor-whey negative tests as compared with two instances of blood negative-whey positive tests (ratio of 10 to one). As mentioned earlier, *Brucella* were isolated from eight of the 21 blood reactor-whey negative animals found in the second study.

**TABLE VI**

Blood and Whey Tests on 1,289 Cows in 51 Blood Test Reactor Herds and in 22 Ring Test Suspicious-Blood Test Negative or Suspicious Herds

<table>
<thead>
<tr>
<th>Blood Test Classification</th>
<th>No. of Animals</th>
<th>Whey Test Classification—No. Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative (— or 1+)</td>
</tr>
<tr>
<td>Negative</td>
<td>1,113</td>
<td>1,108</td>
</tr>
<tr>
<td></td>
<td>86%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Suspicious</td>
<td>88*</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>81%</td>
</tr>
<tr>
<td>Reactor</td>
<td>88</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>24%</td>
</tr>
</tbody>
</table>

* Includes 5 calf-vaccinated animals (±100 serum titers and negative whey test reactions).

Aseptic milk samples for culturing were obtained from 28 of 31 blood test negative or suspicious animals with negative to 5+ whey reactions. The results of the culture studies are given in Table VII. The five calf-vaccinated animals with ±100 blood titers listed in Table VII were treated as reactor animals in the report of the second study (2). *Brucella* were isolated from five animals, all of which had blood titers in the suspicious range and one a negative whey test reaction. Unfortunately, one of the two blood negative-whey positive animals found in this study was sold before the farm was revisited to collect aseptic milk samples for culturing.

The results of the first blood retest (30-90 days) were tabulated for as many animals as possible in the blood negative or suspicious titer range with negative to 5+ whey reactions, to obtain additional information regarding possible infection in animals of this type. The data are given in Table VIII.
TABLE VII

Brucella Isolation Studies on Blood Test Negative or Suspicious Animals With Negative to 5+ Whey Test Reactions

<table>
<thead>
<tr>
<th>Whey Test—No. Animals</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>— 50</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1 50</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1*</td>
</tr>
<tr>
<td>+ 50</td>
<td>0</td>
<td>0</td>
<td>2*</td>
<td>2</td>
<td>3*</td>
</tr>
<tr>
<td>1 100</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>+100**</td>
<td>5*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No. Cult. 28

No. Isol. 5

* Brucella isolated from one animal with these serum and whey reactions.
** Calf-vaccinated animals.

TABLE VIII

First Retest of Blood Test Negative or Suspicious Animals (Whey Test Negative to 5+) in Reactor Herds

<table>
<thead>
<tr>
<th>Serum Titer</th>
<th>Whey Reactions</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>— 50</td>
<td>1+</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1</td>
</tr>
<tr>
<td>1 50</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>+ 50</td>
<td>—</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>1</td>
</tr>
<tr>
<td>1 100</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1</td>
</tr>
</tbody>
</table>

Total 48

<table>
<thead>
<tr>
<th>First Blood Retest Results</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

20 22 6*

* Titer for these animals were: one + 100, two I 200, and three + 200 or higher.
SUMMARY FOR THE DIAGNOSIS OF BOVINE BRUCELLOSIS

Of the 43 blood test suspicious animals that were negative to the whey test (— or 1+), six were diagnosed later (30-90 days) as reactors on the basis of the first blood retest results. The four whey test suspicious or positive (2+ to 5+) animals, on which Brucella isolation results were negative, were diagnosed later as negative to the blood test.

DISCUSSION

The results of these two studies indicate that the whey plate test is not satisfactory as an official diagnostic test for bovine brucellosis under the conditions prevailing in Minnesota.

This conclusion is based on the following facts:

1. Negative whey plate tests were obtained on 62 (21 percent) of the 292 blood test reactor animals studied.
2. Brucella organisms were isolated from 15 (24 percent) of the 62 whey negative-blood reactor animals.
3. Blood and whey plate tests on all lactating animals (1,289) in 51 blood test reactor herds and in 22 ring test suspicious herds that were classified as negative or suspicious to the blood test, disclosed 21 instances of blood reactor-whey negative animals as compared with two instances of blood negative-whey positive animals.

The Brucella isolation attempts on single milk samples from 50 of the 62 blood reactor-whey negative animals proved negative. Various tissues from eight of the 50 animals were cultured, resulting in Brucella isolations from three animals so cultured. There is no way of knowing what the number of additional Brucella isolations might have been had tissues been cultured from the remaining 42 animals on which the milk culturing proved negative. Had this culturing been done, it would seem logical to believe that a number of additional isolations might have been made. The culturing of tissues from eight animals represents a very limited number of animals, but the three additional Brucella isolations which were made indicate rather definitely that a critical evaluation of the whey plate test as an official diagnostic test must include the culturing of various tissues of the animals in addition to the milk.

SUMMARY

Blood and whey plate tests were made on 292 blood test reactor animals. Negative whey tests were obtained on 62 (21 percent) of the 292 animals. Brucella were isolated from 15 (24 percent) of the 62 whey test negative animals.

Blood and whey plate tests were made on all milk producing animals (1,289) in 51 blood test reactor herds containing 88 reactor animals and in 22 ring test suspicious herds that were classified as negative or suspicious to
the blood test. These tests disclosed 21 blood reactor-whey negative animals as compared with two blood negative-whey positive animals.

The studies indicate that the whey plate test is not satisfactory as an official diagnostic test for bovine brucellosis under the conditions present in Minnesota.

REFERENCES


Prelude to Report

We will now call on Dr. R. W. Smith, of Concord, New Hampshire, for the report of the Committee on Brucellosis.

Doctor Smith: Before I read this report, Mr. President, I would like to thank everyone on the Committee and all those who participated in the hearings on Monday, Tuesday, and Wednesday for their thoughts and information and for airing the troubles they are having back home, which are not as many as you might think. It all adds a great deal to the program of brucellosis eradication. Speaking for the Committee, may I say we appreciate your interest in this subject.

Report of Committee on Brucellosis

R. W. Smith, Chairman, Concord, New Hampshire; F. G. Buzzell, Augusta, Maine; J. L. George, Lincoln, Nebraska; David A. Hill, Columbus, Ohio; W. D. Knox, Fort Atkinson, Wisconsin; A. K. Kuttler, Salt Lake City, Utah; C. A. Manthei, Beltsville, Maryland; C. K. Mingle, Riverdale, Maryland; H. H. Payne, El Campo, Texas; L. A. Rosner, Jefferson City, Missouri; A. P. Schneider, Boise, Idaho; F. L. Schneider, Albuquerque, New Mexico; J. V. Smith, Hartford, Connecticut; J. E. Stuart, Sacramento, California; K. Wells, Ottawa, Canada; R. L. West, St. Paul, Minnesota; J. F. Wilbur, Helena, Montana.

Mr. President, members of the Association, and invited guests:

One year ago in Chicago your Committee on Brucellosis, in its report to this Association, briefly reviewed the progress made in the brucellosis eradication program down through the years. Special reference was made to the 1947 report, the year that our present program was officially approved by this Association and by the then, Bureau of Animal Industry, United States Department of Agriculture. It might be well to record here that at that time, 1947, there was but one state which had qualified as modified certified brucellosis-free, namely, North Carolina. In 1949, New Hampshire joined the modified certified list, to be followed two years later by the State of Maine. In the years that followed, the eradication program received special attention in a number of states, and in 1956 two more states joined this select list, namely, Wisconsin, and Washington. We are pleased to report to you now, as has been reported to you earlier this afternoon by Dr. C. K. Mingle, that since we met a year ago the States of Minnesota, Delaware, Connecticut and Vermont have received their brucellosis-free certificates.

While the record shows that definite progress has been made in the past and all indications are to the effect that many more states will meet their goal during the next two or three years, it is evident that every effort must be made in the eradication program if we are to meet the goal of a modified
certified United States by 1960. While our prime object must be to test all animals within a given state and to reduce the infection to the percentages required to qualify as a modified certified brucellosis-free area, we must not neglect nor lose sight of the fact that continued testing and eternal vigilance be exercised in the states which have already qualified, if we are to hold the line and advance our fronts in new territories.

Since we arrived here the first of the week, your Committee has held hearings on Monday, Tuesday and Wednesday evenings. We have endeavored to hear every bit of evidence that our members and scientists have desired to present to us, and I might say that many of those who have appeared before your Committee in hearings have been ranchers, dairymen and other individuals interested in brucellosis eradication. We have listened and studied all testimony very carefully, and we have endeavored to include in this report any and all amendments and recommendations that we as a committee are convinced will be of material benefit and help in carrying out the program of brucellosis eradication during the years ahead. Your Committee realizes that those working in the field on this program must be guided to a great extent by the information furnished them by our scientific, and research workers. If we are going to continue to fight this battle of disease eradication, we also realize that we must have the knowledge and experience of those working in the field, whose job it is to put into effect the knowledge that we have gleaned down through the years relative to the disease itself, and to the methods that we employ in eradicating brucellosis from our livestock population.

It is the belief of your Committee that if the program of brucellosis eradication is to be brought to a successful conclusion the accelerated program, as now in effect, must be continued without interruption. To accomplish this, sufficient Federal and State funds must be provided.

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

1. Additional progress reports of research on the Whey Plate Test were made at the committee meetings and it is the opinion of this Committee that further studies are necessary before the value of the test can be ascertained.

2. It is recommended that further research be conducted to determine standards in conducting and interpreting the B.R.T., or Milk Ring Test.

3. Measures should be instituted by the Agricultural Research Service to restrict the use of the "shield and V" vaccination tattoo to official work performed under the supervision of licensed, accredited veterinarians.

4. In the interest of eradication of brucellosis in swine, the Committee invites the National Swine Council to canvas its membership regarding desired Brucellosis Control procedures with particular emphasis on suggested regulations controlling the intrastate and interstate movement of swine which may
be infected. The recommendations should be submitted to this Committee prior to May 1, 1958.

5. Your Committee recommends the following changed to the Uniform Methods as presented last year:
   (a) Part I, Definitions, add the definition of "Range" and "Semi-Range Areas."

   "Range Area"
   An area in which all cattle are maintained on natural forage and/or browse during the entire calendar year.

   "Semi-Range Area"
   An area in which all cattle are maintained on natural forage and/or browse during the entire calendar year with occasional supplemental feeding.

   Exception: When in the judgment of the State and Federal Livestock Disease Control Officials local factors prevail which are not conducive to the eradication of brucellosis, such areas need not be classed as Range or Semi-Range.

   (b) Part III, Section II, Par. B, change paragraph to read, "If the retest of a certified herd or of animals from such a herd reveals one reactor, the herd may be recertified on the result of one negative herd test conducted not earlier than sixty (60) days following the removal of the reactor."

   (c) Part III, Section II, Par. E, line 5, delete, "classed as infected and tested," insert, "retested."

   (d) Part III, Section I, Par. C (2), line five, delete "practical," insert, "practiced."

   (e) Part IV, Section I, add paragraph to read,

   "F. Bulls and female cattle being held under quarantine for feeding purposes separate and apart from dairy or breeding cattle, may be exempted from test provided such cattle are sold for immediate slaughter under permit from the appropriate Livestock Sanitary Official at the end of the feeding period."

   (f) Part IV, Section II, Par. B, add the following sentence:

   "The herds tested to equal the 20 percent shall not include the same 20 percent previously tested for this purpose."

   (g) Part IV, Section II, add paragraph to read, "F. Bulls and female cattle being held under quarantine for feeding purposes separate and apart from dairy or breeding cattle, may be exempted from test provided such cattle are sold for immediate slaughter under permit from the appropriate Livestock Sanitary Official at the end of the feeding period."

   (h) Part IV, Section III, Par. E, line one, after "age," insert "including Official Vaccinates over thirty (30) months of age."

   (i) Part IV, Section III, Par. E, line five, delete, "60," insert "90."
REPORT OF THE REPRESENTATIVE 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 was held at the Sheraton Hotel, Chicago, Illinois on Thursday, February 14, 1957.

Out of a total membership of 29 organizations, 22 were represented and seven were not represented. The roll of Member Organizations was called as follows:

- American Agricultural Editors' Association Present
- American Farm Bureau Federation Present
- American Meat Institute Present
- American Medical Association Not Present
- American National Livestock Association—Changed to: American National Cattlemen’s Association Present
- American Public Health Association Present
- American Veterinary Medical Association Present
- Association of Land-Grant Colleges and Universities Present
- Department of Agriculture Not Present
- The Dairy Industry Committee Present
- Livestock Conservation, Inc. Present
- National Association of Artificial Breeders Present
- The National Association of Swine Records Not Present
- National Beef Breed Association Present
- National Farmers Union Not Present
- The National Grange Not Present
- National Independent Meat Packers Association Present
- National Live Stock and Meat Board Present
- The National Milk Producers Federation Not Present
- National Research Council Present
- National Wool Growers Association Present
- Purebred Dairy Cattle Association Present
- Texas and Southwestern Cattle Raisers Association Present
- United States Department of Agriculture Extension Service Present
- United States Livestock Sanitary Association Present
- United States Public Health Service Present
- Western States Meat Packers Association Present
- Forest Service, Agricultural Research Service, United States Department of Agriculture Present
- Bureau of Land Management, United States Department of Interior Present

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DELEGATE TO THE NATIONAL BRUCELLOSIS COMMITTEE

The roll call of all present, including guests, as well as delegates was as follows:

Herman C. Aaberg  American Farm Bureau Federation
Robert J. Anderson  Animal Disease Erad. Branch, A.R.S., United States Department of Agriculture
Thomas F. Arnold  American National Cattlemens Association
Allan C. Atlason  American Shorthorn Breeders Association
Richard E. Burleson  Federal Extension Service, United States Department of Agriculture
Hugh S. Cameron  School of Veterinary Medicine, U. of California
Acord Cantwell  Indiana Farm Bureau, Inc.
Ray L. Cuff  Livestock Conservation, Inc., Kansas City
J. W. Cunkelman  Swift & Company
Fred C. Driver  United States A.D.E.B., St. Paul
Willard R. Evans  National Brown Swiss Association
K. K. Heideman  American Farm Bureau Federation
O. W. Hubbard  Esskay Company
L. M. Hutchings  National Research Council
C. A. Joy  United States Forest Service
H. E. Kingman, Jr.  American Veterinary Medical Association
A. K. Kuttler  State and Federal Departments of Agriculture
E. J. Leenhouts  New York Central
Blaine Liljenquist  Western States Meat Packers Association
J. L. McAuliff  American Veterinary Medical Association
S. H. McNutt  Association of Land Grant Colleges
C. A. Manthei  Agricultural Research Service, United States Department of Agriculture
Tom Marshall  West Chicago Stockyards
C. K. Mingle  Agricultural Research Service, United States Department of Agriculture
Joe G. Mingle  Texas & Southwestern Cattle Raiser's Assn.
Keith E. Myers  National Swine Growers Council
Carl F. Neumann  National Live Stock & Meat Board
H. S. Nicol  Iowa Farm Bureau Federation
Fred Olander  Kansas City Livestock Exchange
George E. Parsons  Michigan State University
A. G. Pickett  Kansas Livestock Association
R. K. Pierson  Bureau of Land Management
M. H. Roepke  University of Minnesota
Edwin Sanderson  Texas Farm Bureau
E. E. Saulmon  Animal Disease Eradication Branch, United States Department of Agriculture
William Schwab  Armour & Company
C. G. Scruggs  American Agricultural Editors Association
Fred M. Shigley  Animal Disease Eradication Branch, A.R.S., United States Department of Agriculture
B. T. Simms  Livestock Research, United States Department of Agriculture
R. W. Smith  United States Livestock Sanitary Association
Newly elected officers were:

Thomas F. Arnold, Chairman
J. F. Cavanaugh, First Vice-Chairman
Sam H. McNutt, Second Vice-Chairman
Wallace N. Dudney, Secretary

Executive Committee

Allan Atlason
Richard E. Burleson
J. F. Cavanaugh
B. R. Evans
W. D. Knox
John L. McAuliff
Sam H. McNutt
Carl Neumann
Charles Scruggs

Board of Directors

Terms Expire 1958
D. L. Urschel
T. F. Arnold
Paul Zillman
John Killick
Walter Winn
Ray Willoughby
Charles Scruggs

Terms Expire 1959
Herman C. Aaberg
Allan Atlason
Walter Hunnicut
J. H. Webb
B. R. Evans
Carl Neumann
W. A. Wentworth
Charles A. Joy
Edward Woozley

Terms Expire 1960
R. J. Anderson
Richard E. Burleson
J. F. Cavanaugh
L. M. Hutchings
W. D. Knox
John L. McAuliff
Sam H. McNutt
Keith Myers
R. W. Smith
The following program was presented, and was not only very instructive, but interesting to all present:

10:00 A.M. Call to Order: Herman C. Aaberg, Chairman

Business Meeting:
Subcommittee reports (10 to 15 minutes each):

Information and Education:
Dr. Chas. E. Bell, Jr., Chief A.I.B., F.E.S., United States Department of Agriculture

Research:

Public Health:
Dr. J. H. Steele, Chief, Vet. Public Health Service, Atlanta, Georgia

11:00 A.M. Progress Report:
Dr. C. K. Mingle, Animal Disease Eradication Branch, A.R.S., United States Department of Agriculture

11:20 A.M. Testing Dry and Cull Cattle:
Dr. A. E. Crouse, Supervisor, Dairy and Livestock Division, Washington State Department of Agriculture, Olympia, Washington

11:40 A.M. Testing Dry and Cull Cattle:
Dr. F. M. Shigley, Vet. in Charge, Animal Disease Eradication Branch, A.R.S., United States Department of Agriculture, Olympia, Washington

12:00 Noon Luncheon

1:00 P.M. Report of Nominating Committee and Election of Officers

1:30 P.M. California Research on Whey Test:
Dr. Hugh Cameron, Prof. of Vet. Science, U. of C., Davis, California

2:00 P.M. Minnesota Research on Whey Test:

2:30 P.M. Swine Brucellosis:
Mr. Keith Myers, Exec. Secy., Natl. Swine Council, Grundy Center, Iowa

3:00 P.M. Brucellosis Problems on Public Grazing Lands:
Mr. C. A. Joy, Chief, Div. of Range Management, Forest Service, United States Department of Agriculture

3:15 P.M. Brucellosis Problems on Public Grazing Lands:
Mr. Edward Woozley, Director, Bur. of Land Mgmt., Dept. of Interior

3:30 P.M. Proposed Alternate Method of Certifying Range and Semi-Range Areas:
Dr. R. W. Smith, State Veterinarian, State Hse., Concord, New Hampshire

3:45 P.M. Interstate Regulations:
Dr. R. J. Anderson, Chief, Animal Disease Eradication Branch, A.R.S., United States Department of Agriculture, Washington, D.C.

The meeting adjourned at 4:00 P.M.
Chairman Aaberg's report and recommendations contained quite a bit of information that should be of interest to the members of the United States Livestock Sanitary Association, and we are including his remarks in our report which are as follows:

Chmn. Aaberg: There are about five member organizations that have not been present in the last few years and according to action taken in 1953, they are no longer qualified members. I'll read those in that category:
1. The National Association of Swine Records.
2. National Farmers Union.
3. The National Grange.
4. The National Milk Producers Federation.

I should like at this time to make a few remarks on this problem and on the work of your National Committee.

In reviewing the objectives set forth in the Articles of Incorporation of the National Brucellosis Committee, I find this statement: "To promote, aid or engage in educational activities and scientific research in the field of brucellosis control, including the coordination and focusing of all the forces of organized agriculture in the United States; to combat through every practical device the brucellosis threat to the nation's health and food supply."

It is gratifying to note that significant progress has been made in achieving these objectives over the years. Significant progress has been made in the past year toward our goal to eradicate brucellosis in the United States not later than 1960. Now that the States of Washington, Wisconsin and more recently, Delaware, have obtained the certified, modified, brucellosis-free status along with the States of North Carolina, Maine and New Hampshire, and the fact that many other states are well on the way to achieving this goal, we can take considerable pride in this significant progress.

Dr. C. K. Mingle, Chief, Brucellosis Eradication Section, Animal Disease Eradication, U.S. Department of Agriculture, will report in considerable detail on progress being made in each State, as well as nationally, to eradicate brucellosis.

We are very much encouraged by the growing support of all livestock and dairy groups and others having an interest in this program. We are certain that the livestock and dairy industry appreciates the financial support provided by the Congress and by many state legislatures under the stepped-up brucellosis eradication program. This would not have carried except for a sound program and real interest on the part of livestock and dairy men.

Adoption by the United States Department of Agriculture of uniform interstate regulations governing the movement of cattle under the brucellosis program which was made effective January 1, 1957, appears to be generally acceptable when fully understood. No doubt minor revisions will be neces-
sary. However, the administrators do have considerable leeway to work out special cases.

It will be remembered that this Committee last year unanimously approved the proposed regulations which were drafted upon the insistence and with the full cooperation of the official representatives of the major livestock and dairy associations. The regulations are designed to prevent the spread of brucellosis while at the same time avoid unnecessary hardships and to promote the free movement of all livestock not infected or exposed to the disease.

You will note from the agenda drawn up by your Program Committee under the able leadership of George Parsons of Michigan, that attention is being given to new techniques of detecting infected animals under both range and farm conditions. We need to always keep an open mind to new and better methods of dealing with this problem.

By action of this Committee, representatives of the Federal Government having responsibility for supervising grazing on public lands have been added as members of the Committee. The primary purpose of this action was to facilitate the eradication of brucellosis amongst cattle grazing upon the public lands. These lands represent approximately one-half of the area of the 11 western states. We are pleased to have the representatives of these two important public agencies here with us today to discuss problems in connection with the eradication of brucellosis.

Many of you have recognized the importance of stepping up the program to eradicate brucellosis in swine as well as in cattle. We have, therefore, given emphasis to this problem here today.

I should like to suggest that the National Brucellosis Committee consider the advisability of setting up a special committee to study the Articles of Incorporation and By-Laws to see whether or not changes should be made in the light of new conditions. As an example, consideration might well be given to either broadening the work of the Committee to include other livestock diseases or to suggest that special sub-committees of Livestock Conservation, Inc., be established for this purpose. Some of our states are setting up Animal Health Committees, rather than to confine the activities of the committee to the problem of eradicating a single disease, such as brucellosis.

We are also continuing to experience some difficulty in keeping our membership up-to-date. It will be recalled that in 1953 action was taken as follows:

"Attention was called to the fact that certain member organizations had failed to be represented at any of the meetings thus far, making it difficult to obtain a quorum for meetings. In order to correct this situation, motion was made by Wentworth, seconded by Shannon, and carried, that member organizations not represented at two or more consecutive annual meetings, that the President write the representative and the member organization to ascertain whether or not they wish to continue their membership. If not, they should be discontinued."
I am advised that the following three member organizations have not been represented at either the 1955 or 1956 annual meetings:

1. National Association of Artificial Breeders.
2. National Association of Swine Record Associations.

According to the action taken in 1953, we are required to notify the individual representatives, as well as the organizations, that they are no longer qualified as members of the National Brucellosis Committee.

I should like to state in closing that although we have established as a goal the eradication of brucellosis by 1960, and are making considerable progress in achieving this goal, the problem of complete eradication and of protecting healthy animals from re-infection will remain for an indefinite period. As long as there are areas of infection, there’s also the danger of re-infection of healthy herds. Therefore, calfhood vaccination in such areas will undoubtedly be necessary for several years. We must keep this in mind and not become complacent in our efforts to bring about complete eradication.

I want to express my appreciation to those who have served on the various sub-committees and particularly to those who have served as chairmen of such committees. I want, also, to express appreciation in behalf of the National Brucellosis Committee to all those who are participating in the program.

All other papers and reports of committees have been printed in the 1957 proceedings of the annual meeting of the National Brucellosis Committee, and I would suggest that anyone who is interested in any one or all of the papers as presented, to contact Mr. Wallace N. Dudney, Secretary, 405 Exchange Building, Chicago 9, Illinois, and you no doubt can receive a copy of the proceedings.

The following day your representative attended the annual meeting of Livestock Conservation, of which your delegate is a member of the Executive Committee.

Respectfully submitted,

ROBINSON W. SMITH, D.V.M.
CONTROL OF BOVINE LEPTOSPIROSIS


Since the first report of bovine leptospirosis by Jungherr (1) in 1944, this disease has become recognized in most all the cattle-producing areas of the United States. Due to the great variability of bovine leptospirosis, it is often difficult to evaluate its significance and even recognize its presence. We do have a United States Department of Agriculture estimate of a 112 million dollar annual loss (2). Owners readily recognize the economic importance of abortion storms, feedlot losses and decreased milk production, but the general unthriftiness may be overlooked.

I. Effectiveness of Bacterins

The economic importance of leptospirosis in the eastern states resulted in the development of an egg-propagated L. pomona bacterin by York and Baker (3), which protected calves challenged two to six weeks after vaccination. York and Brueckner (4) reported that leptospirosis was favorably controlled by the use of this bacterin in a number of herds. Stoenner (5) pointed out that evaluation of bacterins was often quite difficult in field outbreaks, since a large part of the herd may be infected before vaccination can be carried out.

A culture bacterin was found by Brown et al. (6) to protect calves challenged three weeks after vaccination. These workers also demonstrated antibodies in vaccinated calves for at least five months. Later Burnstein et al. (7) reported that the culture bacterin protected cattle against challenge six, 12 and 14 months after vaccination. Hoag and Bell (8) have described a soluble antigen which protected calves when challenged two months later.

Gillespie et al. (9), employing three different bacterins†, reported that eight- to 12-month-old cattle acquired a high level of resistance which per-

* From the Washington Agricultural Experiment Station, Dept. of Veterinary Science, Dept. of Veterinary Microbiology and Dept. of Veterinary Clinical Medicine and Surgery, State College of Washington, Pullman. Scientific Paper No. 1677, Project No. 1176.

† Bacterins included an egg-propagated product (Leptogen), an experimental culture bacterin developed by American Cyanamid Co. and one prepared by Gillespie at Wash. State College.
sisted for at least six to eight months (Table 1). Over half of another group of cattle showed moderate resistance to challenge 15 months after vaccination. Young calves, vaccinated at one or two months of age, failed to demonstrate a satisfactory immunity when challenged six to eight months later. Further studies (Tables 1 and 2) indicated that 8 of 12 cattle were still resistant 18 to 20 months after vaccination. In another experiment all the calves vaccinated at three to five months of age were protected against leptospiruria.

Ideally, a bacterin should afford protection against the appearance of fever, leptospiruria or infection titers. In these exposure studies, none of the vaccinates* became febrile, but 70 per cent (15/21) of the controls developed fevers. However, about a third of the vaccinates (14/45) developed infection titers as compared with the 100 percent occurrence in the controls (21/21). Usually the infection titers of the "reacting" vaccinates were one or two 10-fold dilutions less than that of the controls. Half of the vaccinates with infection titers (7/14) developed leptospiruria, while 85 percent of the controls (18/21) became shedders. It is interesting to note that most of the vaccinates with leptospiruria (5/7) shed at a rate which was detectable only by hamster or guinea pig inoculation. Since 80 percent of the controls (16/21) were moderate to heavy shedders, the bacterin appeared to have exerted a protective effect even on those vaccinates which became shedders.

All cattle were challenged by the instillation of the diluted urine into the eyes and nostrils over a period of six days. The urine was collected from a shedding cow(s) (contained about 50 million leptospirae/ml.), diluted 1:2 or more with water containing suspended kaolin. Leptospirae-containing urine was also placed in the drinking water for nine days. Obviously, the number of organisms in each challenge-exposure could not be standardized with this procedure. We feel, however, that the method of exposure was certainly severe enough since 100 percent of the controls became infected. The level of exposure for Group IV may have been more severe than for Group V in view of the larger ratio of unprotected vaccinates (Table 2).

Vaccinates and controls were quarantined in holding pens located on high ground with no drainage or overflow from areas occupied by domestic animals. Exposure trials were conducted in isolation units, where animals were held until completion of experimental studies.

Gillespie (unpublished data) has shown that bacterins stimulate the production of protective antibodies (Table 3). These antibodies are directly related to the animal's resistance upon challenge.

* Refers to the cattle vaccinated when three months or more in age.
TABLE 1
Evaluation of *L. pomona* Bacterins*

<table>
<thead>
<tr>
<th>Challenge Groups and Age When Vaccinated</th>
<th>Vaccination-Challenge Interval</th>
<th>Vaccine</th>
<th>No. 1*</th>
<th>No. 2**</th>
<th>WSC***</th>
<th>Total Vaccinates</th>
<th>Controls</th>
<th>Vaccinates Resisting Infection (Totals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (6-8 mo. heifers)</td>
<td>7½-8½ Mo.</td>
<td></td>
<td>0/5</td>
<td>1/5</td>
<td>0/2</td>
<td>1/12</td>
<td>5/5</td>
<td>11/12</td>
</tr>
<tr>
<td>Groups II and III (1-2 mo. calves)</td>
<td>6½-8</td>
<td></td>
<td>6/8</td>
<td>7/9</td>
<td></td>
<td>13/17</td>
<td>9/9</td>
<td>4/17</td>
</tr>
<tr>
<td>Group IV (6-8 mo. heifers)</td>
<td>13-15</td>
<td></td>
<td>4/5</td>
<td>3/5</td>
<td>1/2</td>
<td>8/12</td>
<td>5/5</td>
<td>4/12</td>
</tr>
<tr>
<td>Group V (6-8 mo. heifers)</td>
<td>18-20</td>
<td></td>
<td>1/5</td>
<td>3/5</td>
<td>0/2</td>
<td>4/12</td>
<td>6/6</td>
<td>8/12</td>
</tr>
<tr>
<td>Group VI (3-4 mo. calves)</td>
<td>13</td>
<td></td>
<td>0/5</td>
<td></td>
<td>1/4</td>
<td>1/9</td>
<td>5/5</td>
<td>8/9</td>
</tr>
</tbody>
</table>

* From manuscript by Gillespie and Kenzy.

* Leptogen.

** An experimental product prepared by Lederle Laboratories.

*** An experimental product prepared in the College of Veterinary Medicine, State College of Washington.
TABLE 2

Effect of Vaccination with Bacterins on the Rate of Shedding

<table>
<thead>
<tr>
<th>Challenge Groups and Age When Vaccinated</th>
<th>Vaccination-Challenge Interval (Mo.)</th>
<th>Unprotected Vaccinates</th>
<th>Ratio of Shedders to Those Exposed</th>
<th>Vaccinates</th>
<th>Limits to Heavy Shedding</th>
<th>Total Shedders</th>
<th>Controls</th>
<th>Limited Shedding</th>
<th>Moderate to Heavy Shedding</th>
<th>Total Shedders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (6-8 mo. heifers)</td>
<td>7⅓-8⅓</td>
<td>1/12a</td>
<td>1/12</td>
<td>0/12</td>
<td>1/12</td>
<td>1/5</td>
<td>3/5</td>
<td>4/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (1-2 mo. calves)</td>
<td>6½</td>
<td>7/9</td>
<td>2/9</td>
<td>3/9</td>
<td>5/9</td>
<td>0/6</td>
<td>5/6</td>
<td>5/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III (1-2 mo. calves)</td>
<td>8</td>
<td>6/8</td>
<td>0/8</td>
<td>4/8</td>
<td>4/8</td>
<td>0/3</td>
<td>3/3</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV (6-8 mo. heifers)</td>
<td>13-15</td>
<td>8/12</td>
<td>4/12</td>
<td>1/12</td>
<td>5/12</td>
<td>1/5</td>
<td>3/5</td>
<td>4/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V (6-8 mo. heifers)</td>
<td>18-20</td>
<td>4/12</td>
<td>0/12</td>
<td>1/12</td>
<td>1/12</td>
<td>0/6</td>
<td>5/6</td>
<td>5/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group VI (3-5 mo. calves)</td>
<td>13</td>
<td>1/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/5</td>
<td>5/5</td>
<td>5/5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Ratio of unprotected to total number vaccinated.
* Shedding detected only by laboratory-animal inoculation.
* Shedding detected by darkfield examination of urine.
CONTROL OF BOVINE LEPTOSPIROSIS

TABLE 3
The Relation of Protective Antibodies to the Resistance of Vaccinates

<table>
<thead>
<tr>
<th>Vaccinate Group</th>
<th>Number of Cattle</th>
<th>P. A.* Present</th>
<th>Infected on Challenge</th>
<th>Evidence of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1a</td>
<td>5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>WSCa</td>
<td>4</td>
<td>3/4</td>
<td>1/4(^b)</td>
<td>1/4</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>0/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

* Protective antibodies titrated in 2-day-old chicks.
** Positive agglutination-lysis reaction.
\(a\) Vaccinates received a single dose of bacterin at three to five months of age.
\(b\) This animal had no protective antibodies.

II. Preliminary Studies of an Egg-passaged Strain of
L. pomona as an Immunizing Agent

Although there are many favorable field reports concerning the use of bacterins, we have observed a number of very definite failures. In one case a 600-cow herd experienced over 40 successive abortions without a viable calf. These losses, which continued for over a month following vaccination, decreased in number after a change of pastures and feeding areas. Leptospirae were isolated from surface waters and from 18 of 20 aborting cows through urine inoculation of guinea pigs. Other recognized causes of abortion were eliminated. Such observations restimulated our interest in Reinhard's report (1) concerning the study of a 100-egg-passaged (E-P) strain of L. pomona which failed to produce leptospiruria or fever in cattle, and produced an immunity which resisted challenge 17 months later.

A 500-egg-passaged (E-P) strain of L. pomona was obtained from Dr. York for studies to determine its pathogenicity. Limited numbers of animals (Table 4) were inoculated with the 503d egg-passage and followed for clinical evidence of disease. Other than a fever for one to two days in some of the cattle, no evidence of illness was found. The very rapid development of antibodies within eight days in animals more than two months of age may prove effective in the rapid control of field outbreaks. The two-month-old calves do not respond as well as the older animals, but antibodies were still present 300 days after inoculation. Our experience would indicate that these cattle are immune. We plan to challenge these animals later when antibody levels have disappeared.

Leptospiruria (detected only by inoculation of urine into the hamster) occurred at a low rate for one to two weeks in half of the test cattle. In the case of the four and six year old cows, shedding persisted much longer than in the younger animals.
### TABLE 4

The Effect of Living Egg-passaged* *Leptospira pomona* on Cattle (Preliminary trial)

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (Mos.)</th>
<th>Blood Titers b</th>
<th>Leptospiruria</th>
<th>Fever e</th>
<th>Kidney Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days Following Inoculation</td>
<td></td>
<td>D/F c</td>
<td>Lab. Animal Inoculation d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>28</td>
<td>77</td>
<td>300</td>
</tr>
<tr>
<td>176</td>
<td>12</td>
<td>+10⁻³</td>
<td>+10⁻³</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
</tr>
<tr>
<td>191</td>
<td>6</td>
<td>+10⁻³</td>
<td>+10⁻³</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
</tr>
<tr>
<td>194</td>
<td>6</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
</tr>
<tr>
<td>196</td>
<td>6</td>
<td>+10⁻³</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
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<tr>
<td>207</td>
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<td>±10⁻¹</td>
</tr>
<tr>
<td>210</td>
<td>2</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
<td>+10⁻¹</td>
<td>±10⁻¹</td>
</tr>
<tr>
<td>230</td>
<td>48</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
<td>±10⁻²</td>
</tr>
<tr>
<td>237</td>
<td>72</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
<td>+10⁻²</td>
<td>±10⁻²</td>
</tr>
</tbody>
</table>

---

* 1.5 billion organisms of the 503rd egg passage were given SQ just posterior to the scapula.

b Agglutination-lysis tests.

c Darkfield examination.

d Weeks positive for shedding on the basis of a serologic reaction in either the hamster or guinea pig inoculated with urine.

e Days after inoculation when fever appeared.

f Refers to an agglutination-lysis reaction involving more than 10 per cent but less than 50 per cent of the leptospirae.
The mild nephritis in two of the cattle would suggest that the kidney damage was minimal. Kidney biopsy material from this group of animals failed to produce infection when inoculated into hamsters and was negative on culture.

III. Effect of Virulent Exposure on Egg-passaged L. pomona
Inoculated Calf Groups and on Calf Groups Receiving Bacterin

Further studies were carried out in an attempt to determine how soon an effective immunity developed in cattle inoculated with either the egg-passaged (E-P) strain or a bacterin. Such experimental groups were then given a virulent challenge and observed for evidence of illness and leptospiuria.

The experimental groups and respective treatments are listed below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>E-P culture, two weeks before virulent exposure.</td>
</tr>
<tr>
<td>2</td>
<td>E-P culture, one week before virulent exposure.</td>
</tr>
<tr>
<td>3</td>
<td>E-P culture on the same day as virulent exposure.</td>
</tr>
<tr>
<td>4</td>
<td>E-P culture three days after virulent exposure.</td>
</tr>
<tr>
<td>5</td>
<td>Bacterin two weeks before virulent exposure.</td>
</tr>
<tr>
<td>6</td>
<td>Bacterin, one week before virulent exposure.</td>
</tr>
<tr>
<td>7</td>
<td>Controls, virulent exposure alone.</td>
</tr>
<tr>
<td>8</td>
<td>Controls, E-P culture alone.</td>
</tr>
<tr>
<td>9</td>
<td>Contact controls for Group 8.</td>
</tr>
</tbody>
</table>

From the data in Table 5 we note that the E-P leptospiiae have stimulated the development of a high antibody level by the first week (Groups 1, 2, 3 and 8). One lot of bacterin stimulated low-level demonstrable antibodies during the second week, while the other lot (Group 5) required four weeks. After six months, antibody levels were still demonstrable in all groups, with the lowest level in Group 5.

The degree and duration of leptospiuria were determined through weekly darkfield examination of urine and by weekly inoculation of urine into five week old hamsters which were bled and tested by the agglutination-lysis test for antibodies against L. pomona (Table 6).

Shedding was heavier and more persistent in Groups 4 and 7, which fact suggests that the challenge exposure was adequate. Cattle excreting millions of leptospiiae per ml. of urine (4+) were observed only in these groups. The lot of bacterin used for Group 6 gave much better protection against shedding than the bacterin used in Group 5. There may be limited evidence of some interference between the virulent and E-P strains in the case of Group 3.

a Each group consisted of 4 cattle each except Group 4 with 3.

b One billion organisms of the 508th egg passage, subcultured three days, were given subcutaneously just posterior to the scapula.

c REA strain, isolated from a severe outbreak in a beef herd, was used for virulent exposure. About 275 million leptospiiae were instilled into the nostrils and conjunctival sacs on each of six separate exposures over a period of nine hours.

d Unavoidably, this was a different lot from that used in Group 5.
Table 5. SERUM ANTIBODY LEVELS FOR L. POMONA IN GROUPS OF CATTLE EXPOSED TO VIRULENT AND EGG-PASSAGED STRAINS.

<table>
<thead>
<tr>
<th>GROUP no.</th>
<th>0</th>
<th>10-1</th>
<th>10-2</th>
<th>10-3</th>
<th>10-4</th>
<th>10-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
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<td>2nd Week</td>
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<td>1</td>
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<tr>
<td>3rd Week</td>
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<tr>
<td>6</td>
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</table>

* Based on A-L test using live culture antigen
** Weeks after exposure
Table 6. LEPTOSPIRURIA IN CATTLE EXPOSED TO EGG-PASSAGED AND VIRULENT STRAINS OF L. POMONA

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>WEKS OF SHEDDING FOLLOWING EXPOSURE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>EGG-PASSAGE 2 WKS BEFORE VIRULENT EXP.</td>
<td>ANIMAL NO.</td>
</tr>
<tr>
<td>1</td>
<td>215 216 217 218 219 220 221 222 223 224 225 226</td>
</tr>
<tr>
<td>EGG-PASSAGE 1 WK BEFORE VIRULENT EXP.</td>
<td>227 228 229 230 231 232 233 234 235 236</td>
</tr>
<tr>
<td>EGG-PASSAGE + V. EXP. ON SAME DAY</td>
<td>237 238 239 240 241 242 243 244 245 246</td>
</tr>
<tr>
<td>EGG-PASSAGE 3 DAYS AFTER VIRULENT EXP.</td>
<td>247 248 249 250 251 252 253 254 255 256 257</td>
</tr>
<tr>
<td>BACTERIN 2 WKS BEFORE VIRULENT EXP.</td>
<td>258 259 260 261 262 263 264 265 266 267 268 269</td>
</tr>
<tr>
<td>BACTERIN 1 WK BEFORE VIRULENT EXP.</td>
<td>270 271 272 273 274 275 276 277 278 279 280 281</td>
</tr>
<tr>
<td>CONTROL VIRULENT EXPOSURE ALONE</td>
<td>282 283 284 285 286 287 288 289 290 291 292 293</td>
</tr>
<tr>
<td>CONTROL EGG-PASSAGE ALONE</td>
<td>294 295 296 297 298 299 300 301 302 303 304 305</td>
</tr>
<tr>
<td>CONTACTS WITH EGG-PASSAGE CONTROLS</td>
<td>306 307 308 309 310 311 312 313 314 315 316 317</td>
</tr>
<tr>
<td>PRELIMINARY EGG-PASSAGE</td>
<td>318 319 320 321 322 323 324 325 326 327 328 329</td>
</tr>
</tbody>
</table>

- Shaded block on chart indicates the week in which the urine was found to contain leptospires. Based on the appearance of A-L antibodies in Hamster Sera following inoculation of urine.

**Refers to large numbers of Leptospires shed in the urine.
The shedding in the E-P inoculated Groups 1 and 2 is rather limited, but more prevalent than leptospiruria in the E-P inoculated control Group 8. The leptospiruria in Group 1, which occurred during the first three weeks, was most likely due to the E-P strain, since virulent exposure did not occur until after the second week.

It is interesting to note that no shedders were detected in Group 9, in which one animal developed low level agglutinins for a limited time. Group 9 was held in the same pen with Group 8.

The shedding pattern for the preliminary E-P inoculated group (indicated as Group 10 on Table 6) is added for comparison.

Further information concerning the relative pathogenicity of virulent and E-P strains of *L. pomona* is presented in Table 7. The extent of interstitial nephritis was studied by means of kidney biopsies performed about two months after shedding was no longer demonstrable through inoculation of urine into the hamster. The amount of mononuclear cell infiltrate was arbitrarily graded in relation to the extensive nephritis observed in the renal tissue from the virulent exposure control Group 7. Silver stains were also prepared and studied, but no leptospirae were found.

A rather mild nephritis was present in the E-P inoculated Groups 1 and 2. The 2+- nephritis in one of the Group 8 animals was observed in the only shedder of this group.

Although there were no shedders in bacterin Group 6, the nephritis appeared more extensive than that in bacterin Group 5 where all the animals shed.

Only one of the kidney biopsies was positive for leptospirae on culture. This animal had not shed for 54 days. Cultures of kidney tissue 44 days after the first biopsy were negative. On the basis of similar experience with other renal culture studies, it appears that laboratory-animal inoculations are not as sensitive as culture for detection of leptospirae.

Daily temperatures were taken for two weeks after virulent exposure. Febrile response was most evident in Groups 4 and 7, which suggests that the E-P and the bacterin inoculates had received some protection against the virulent exposure. A mild febrile reaction was observed in one of the four E-P inoculated controls (Group 8).
### TABLE 7
The Effect of Virulent and Egg-passaged Strains of *L. pomona* on Cattle

<table>
<thead>
<tr>
<th>Bovine Group No.</th>
<th>Treatment</th>
<th>Leptospirosis c</th>
<th>Extent of Interstitial Nephritis</th>
<th>Weeks Since Virulent Exposure</th>
<th>Culture of Biopsy</th>
<th>Fever e</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E-P&lt;sup&gt;a&lt;/sup&gt; leptospirae given 2 weeks before V-Exp.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/4</td>
<td>0 1+ 2+ 3+ 4+</td>
<td>14</td>
<td>0/4</td>
<td>1/4-12</td>
</tr>
<tr>
<td>2</td>
<td>E-P leptospirae given 1 week before V-Exp.</td>
<td>2/4</td>
<td>2/4</td>
<td>13</td>
<td>0/4</td>
<td>1/4-4</td>
</tr>
<tr>
<td>3</td>
<td>V-Exp. and E-P leptospiroae the same day</td>
<td>2/4</td>
<td>2/3</td>
<td>12</td>
<td>0/4</td>
<td>1/4-4,8,9,13</td>
</tr>
<tr>
<td>4</td>
<td>V-Exp. given 3 days before E-P leptospiroae</td>
<td>3/3</td>
<td>1/3</td>
<td>12</td>
<td>0/3</td>
<td>1/3-6,2/3-7,8</td>
</tr>
<tr>
<td>5</td>
<td>Bacterin given 2 weeks before V-Exp.</td>
<td>4/4</td>
<td>2/4</td>
<td>12</td>
<td>0/4</td>
<td>1/4-6</td>
</tr>
<tr>
<td>6</td>
<td>Bacterin given 1 week before V-Exp.</td>
<td>0/4</td>
<td>0/4</td>
<td>12</td>
<td>0/4</td>
<td>1/4-7</td>
</tr>
<tr>
<td>7</td>
<td>V-Exp. alone (controls)</td>
<td>4/4</td>
<td>1/4</td>
<td>1/4</td>
<td>2/4</td>
<td>1/4-4,11,12</td>
</tr>
<tr>
<td>8</td>
<td>E-P leptospiroae (controls)</td>
<td>1/4</td>
<td>0/4</td>
<td>1/4-7</td>
<td>0/4</td>
<td>1/4-4,12</td>
</tr>
<tr>
<td>9</td>
<td>Contacts with Group 8</td>
<td>0/4</td>
<td>not studied</td>
<td>1/4-7</td>
<td>0/4</td>
<td></td>
</tr>
</tbody>
</table>

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<sup>a</sup> E-P refers to egg-passaged.

<sup>b</sup> V-Exp. refers to virulent exposure.

<sup>c</sup> Detected either by darkfield or by urine inoculation into hamsters.

<sup>d</sup> An arbitrary evaluation of tissue changes in relation to the reaction produced in the virulent exposure controls.

<sup>e</sup> Ratio of those with fever to those exposed and the day(s) following exposure when fever occurred.
IV. Effectiveness of Antibiotics

Data taken from the studies of Ringen et al. (1955) and Ringen and Bracken (1956) demonstrated that 2.5 mg. of tetracycline per pound of body weight was the minimal effective dose, since the 2.0 mg. level failed to prevent the development of carrier animals.

Five mg. of dihydrostreptomycin per pound of body weight given twice a day not only stopped leptospiruria, but also prevented the shedding from occurring. It appears that dihydrostreptomycin may be of definite immediate aid in preventing the spread of bovine leptospirosis. Certainly the movement of purebred animals into clean herds or upon return from shows would be occasions for antibiotic use where preventive vaccination is not practiced.

Preliminary feeding trials of chlortetracycline of 0.5 mg./pound of body weight show some promise of preventing infection under experimental conditions, whereas a 0.1 mg. level did not prevent the development of the carrier animal (unpublished data, Ringen and Okazaki).

TABLE 8
Chemotherapy in Bovine Leptospirosis
(From Publications by Ringen and Bracken)

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Treatment</th>
<th>Dosage mgms Per Pound Body Weight</th>
<th>No. Giving Serologic Response</th>
<th>Leptospirosis Pre-Treatment</th>
<th>Leptospirosis Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>Control</td>
<td></td>
<td>8</td>
<td>7</td>
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<tr>
<td>3</td>
<td>Terramycin</td>
<td>7 (once daily for 5 days)</td>
<td>3</td>
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<tr>
<td>3</td>
<td>Tetracycline</td>
<td>5 (once daily for 5 days)</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Tetracycline</td>
<td>2.5 (once daily for 5 days)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Tetracycline</td>
<td>2.0 (once daily for 5 days)</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Tetracycline</td>
<td>1.0 (once daily for 5 days)</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Dihydrostreptomycin</td>
<td>5.0 (twice daily for 3 days)</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6b</td>
<td>Dihydrostreptomycin</td>
<td>5.0 (twice daily for 3 days)</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4c</td>
<td>Dihydrostreptomycin</td>
<td>5.0 (twice daily for 3 days)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a One animal, leptospirosis questionable.

b Treatment started 13 days post-exposure.

c Treatment started seven days post-exposure.
CONTROL OF BOVINE LEPTOSPIROSIS

SUMMARY

1. Three L. pomona bacterins were found to be comparable immunizing agents in cattle.

2. Bacterins were found to protect 8 out of 12 “short yearlings” against leptospiruria 18 to 20 months after vaccination.

3. Calves, one to two months of age when inoculated with L. pomona bacterins, developed an unsatisfactory resistance, but those three months or older usually developed a high level of immunity.

4. Although bacterins afforded complete immunity against the appearance of a febrile response in all test cattle three months or older, about 10 percent developed leptospiruria and 30 percent infection titers. Over two-thirds of the vaccinates with leptospiruria shed at levels which were only detectable by inoculation of urine into hamsters.

5. Protective antibodies have been demonstrated in vaccinates that resisted infection on challenge.

6. A strain of L. pomona attenuated through more than 500 egg-passages and evaluated as an immunizing agent, gave a more rapid and higher antibody response in calves than the bacterin. The mild nephritis and low-level shedding observed in these studies may require further attenuation of this strain.

7. Kidney tissue from an eight month old calf, 54 days after shedding had apparently ceased, yielded L. pomona when cultured.

8. Five mg. of dihydrostreptomycin per pound of body weight twice a day not only stopped shedding, but also prevented its occurrence.

REFERENCES


REPORT OF THE COMMITTEE ON LEPTOSPIROSIS


As the members of the United States Livestock Sanitary Association are probably aware, this is the first year that a Committee on Leptospirosis has been appointed. The subject has been touched upon briefly by general committees, and a number of pertinent papers on leptospirosis have been presented to the Association in the past seven years. The existence of leptospirosis in domestic animals in this country was first confirmed in 1944, although histopathological and serological evidence indicates that it undoubtedly was present considerably before that time. The successful isolation of the causative organisms in 1946 stimulated a number of research projects on this subject. As a result, there is now available a rather extensive list of references on almost all phases. Of particular interest are the Proceedings of the Interprofessional Symposium on Leptospirosis, held at the University of Kansas Medical Center in April, 1957, and published by "Veterinary Medicine" in November of this year.

Rather than attempt to present in detail any particular phase of the subject, it was felt that this initial committee report would be of most value in summarizing the information presently available and pointing out areas where additional study appears to be needed. Discussion will be limited to the problem in cattle and swine.

All available evidence indicates that the primary etiological agent of leptospirosis in cattle and swine is *L. pomona*. There have been occasional authenticated cases of infection with *L. canicola* and *L. icterohemorrhagiae*, but these appear to be sporadic. Serological work has occasionally detected antibodies of *L. hebdomadis*. Almost invariably these antibodies have been found in herds known to be infected with *L. pomona*, suggesting a non-specific reaction. Of more interest are the rather large number of herds, especially in the southeastern states, possessing antibodies against *L. sejroe*. In some instances, *L. pomona* has been isolated from such herds; in others, it has not. In spite of numerous attempts, no one has yet succeeded in isolating this species of leptospira, i.e., *L. sejroe*. Consequently, until some other organism is isolated, we must work on the premise that *L. Pomona* is the primary etiological agent of leptospirosis in domestic animals in the United States.

Leptospira, as a group, are organisms which usually localize in the kidney and are excreted in the urine. *L. pomona* is no exception, and the usual method of transmission from a carrier animal to a susceptible animal is by means of infected urine. Similarly, water in streams or pastures con-
taminated with infected urine can serve as means of transmission. No rodents in this country have been incriminated as harboring *L. pomona*, and even if carrier rodents were found, they would probably play a minor role in keeping the disease alive. Cattle have been shown to shed leptospires for as long as two to three months, and swine for as long as eight months, thus capable of acting as the main reservoirs and spreaders of the disease.

The incubation period following contact with infected material ranges from seven to 10 days for both cattle and swine. In cattle, there are few diseases which produce such a wide variety of manifestations, ranging all the way from a very mild, inapparent infection to an extreme fulminating febrile illness with death ensuing. The more important manifestations of the disease are:

1. Cattle under one year of age: An illness ranging from an acute febrile illness with hemoglobinuria and death, to a chronic illness with emaciation and unthriftness. Weight loss may persist for several months, followed by recovery, but in many cases, permanent stunting occurs. In many herds, the major financial loss can be attributed to unthriftness of affected animals.

2. Dairy Cattle: Again, the infection varies from an acute febrile illness with loss of weight, milk production, and possibly hemoglobinuria, to a chronic illness. Death is less frequent in older animals. If cows are infected during the third trimester of pregnancy, abortion is frequently observed.

3. Beef Cattle: Possibly due to the animal husbandry methods, the febrile stage is less frequently observed. Due to the fact that beef cows are often in about the same stage of pregnancy, abortion storms appear to be one of the most common features of the disease.

In swine, *L. pomona* produces no signs of illness in either young or adult pigs. The only effect following infection is abortion near the latter part of gestation, or the birth of dead or weak and dying offspring. This damage to the fetuses occurs only if the sows become infected during pregnancy. The incidence of abortions in a given herd varies considerably, from only a few sows to as high as 100 percent. Indications are that leptospiral infection in swine may be the single most important cause of abortions, or of weak and dying litters.

Leptospirosis in cattle can usually be diagnosed on the basis of an accurate history and careful observation of all the clinical signs available. Abortions strongly suggest leptospirosis, especially in herds where brucellosis can be ruled out. Attempts to isolate the causative agent for routine diagnosis are not practical because of the difficulty in cultivating the leptospires. The use of serological tests is especially valuable, since leptospirosis is primarily a herd problem with not all animals involved at the same time. Since abortions occur two, and often three weeks after the febrile stage of the disease, and antibodies begin to appear 10-14 days after infection, serological tests can be of immediate value in determining the cause of the trouble.
A number of serological tests have been developed and used. All of them have merit, but one of the more recent ones, known as Stoener's method, appears to be the most practical. This method employs a rapid plate test for screening procedures, followed by a capillary tube test to determine the titer of the positive serums. The test has been carefully defined, and commercial antigens are now available for use. The use of this test (or a more superior procedure if one is developed in the future) by all diagnostic laboratories can well be considered the first step in development of control program.

Leptospirosis in swine and cattle has now been observed in every part of the United States, and in Canada. Its importance to the livestock industry undoubtedly varies from area to area. The full impact of this disease cannot be accurately ascertained until adequate disease-reporting systems are developed for veterinary medicine. In areas where serological surveys of cattle have been conducted, the incidence ranged from four to 12 percent. In tests with approximately 18,000 random samples, an average incidence of about 6.5 percent was found. In swine, the incidence of infection appears to be much higher, although extreme variations from two to 27 percent have been observed. There was an average incidence of 13.6 percent in tests of approximately 4,000 swine samples.

Much has been written concerning the treatment of leptospirosis in cattle. Time does not permit any detailed discussion concerning this matter. In general, there is no specific therapy which affords dramatic results. Antibiotic therapy and the usual supportive measures appear to be beneficial. It is more important, in considering leptospirosis, to concern ourselves with preventive measures.

The elimination of carrier animals by treatment with antibiotics is such an expensive procedure at the present time that it has not been given serious consideration in this report. For prevention and control of the disease, reliance must be placed on vaccination. Several commercial vaccines are available which provide satisfactory immunity to prevent disease in cattle for a year to a year and a half. Duration of immunity in swine has not yet been fully determined under field conditions, but protection is afforded for at least six months.

It is recommended that Doctor Kenzy's paper be studied carefully for proper use of vaccines. Some of his data suggests that improved standardization of the existing bacterins might be necessary, although as a whole they are satisfactory for use.

The prevention and control of leptospirosis is, at the present time, considered on a herd basis. In the average commercial dairy, beef, or swine herd that becomes infected with leptospirosis, it is doubtful whether it would be economically feasible to dispose of serologically positive animals. Recovery from infection probably results in lifetime immunity, with no well-documented evidence of serious, long-lasting sequelae, with the exception of continued unthriftiness in young animals.

In such infected herds, however, it is essential that all incoming cattle be vaccinated before purchase, or after purchase to vaccinate and hold apart
from the herd for at least two weeks. Owners of infected pure-bred stock should recognize their moral obligation not to sell these animals for breeding purposes. To get back in the business again, the young stock in a herd should be vaccinated and raised apart from the older stock.

To maintain a leptospirosis-free herd, the most reliable procedure, and the most simple, is the practice routine vaccination. Until further information is available, such vaccination should be repeated every 12-18 months. Immunization procedures should also be followed for all commercial assembled herds, or in any situation where groups of cattle from different sources might mingle together.

To maintain a clean herd without vaccination is a more difficult goal to achieve. In such situations all incoming cattle should be held in strict isolation for at least three weeks, at which time a serological test should be conducted. If the test is positive it is necessary to assume that the animals are potentially spreaders of the disease. Such animals may be given a long course of dihydro streptomycin, or disposed of.

It is felt by the committee that neither is there enough data on all phases of leptospirosis, nor is there real need at the moment to develop a state-wide control program. Emphasis should be placed on control at the individual herd level. It is the responsibility of the veterinarian as a professional individual to acquaint his clients as to the possibilities concerning this disease. It is recognized that many phases of leptospirosis need additional study. It is urged that increased funds be made available for experimental work, not only within the A.R.S., but also for qualified veterinary experimental laboratories. It is also recommended that this committee be enlarged and continued for another year. It may be possible by next year to spell out more specifically steps that might be taken for a control program.

However, it must be pointed out that if the recommendations made above are employed on herd basis, the losses now resulting from L. pomona infection should be significantly reduced.

PRESIDENT GOOD: Thank you, Doctor York.

We have a few minutes for discussion. Are there any questions anyone would like to ask either Doctor York or Doctor Kenzy?

SECRETARY HENDERSHOTT: I would like to ask Doctor York if he thinks the time is ripe for the Committee to bring in a suggested regulatory disease control program for leptospirosis and lay it out 1-2-3-4-5.

DOCTOR YORK: As I indicated in the last paragraph of our report, I certainly think there is enough information available to do that, but obviously it would not be of any value unless a given area or group of individuals appeared interested enough to try to use such a program in their area.

I know of some areas where there is very definitely a desire to try to do that, at least in a given beef production area for example, and I think it would be possible to lay out steps in a sort of general manner that could be followed.
SECRETARY HENDERSHOTT: Can we look to your Committee to do it.

DOCTOR YORK: Yes. We will try.

DOCTOR C. A. MANTHEI [Beltsville, Maryland]: I would like to ask Doctor York a question. When he gave the figures on incidence, was he talking about incidence of the disease, incidence of infection, or incidence of reactions to specific tests?

DOCTOR YORK: The only way you can determine the incidence of a disease in a given herd population or a given cattle or swine population is by trying to test animals on a random basis, rather than just testing herds where you suspect the disease.

Obviously, if they have antibodies they have had the disease at some time and have recovered. Hence, that gives you an idea of what percentage of the animals in an area or in a state have at least been exposed to the illness.

DOCTOR MANTHEI: But there is a difference in whether you are talking about incidence of infection or incidence of exposure or incidence of reaction to a specific test. I think we have all recognized that the test has merit in diagnosing the disease on a herd basis. Is it accurate enough to specifically say, "This animal is infected and this one is not"?

DOCTOR YORK: All the serological test can tell you is that this animal has had the disease at some time. If you have an accurate history of that animal, and if you accumulate that information along with the serological test, then you can tell whether the animal is still an infected animal or not, because of the knowledge we have on how long carrier animals can remain shedders. Obviously, if you know the animal has not been sick or that the herd has not had a disease resembling leptospirosis for several months or a year, then it means that the serological test means that those animals had at some time in their past history had the disease. The serological test is only one tool—one phase of the whole thing.

DOCTOR MANTHEI: We don't know that on random sampling.

DOCTOR YORK: That's right.

VOICE: How long after the cattle are vaccinated will reaction last?

DOCTOR KENZY: We have seen partial reactions of 10 to 100 of the –10 dilution of the sera. Animals will vary. Some of them become completely negative serologically six weeks after they are vaccinated; others will continue to react for a year.

It is interesting that some of those that have a high titer, when you challenge them, will become immune, and others with a very low titer will become infected. It is difficult to generalize the importance of the titer, except that in general the higher the titer, the better the immunity, I believe.

VOICE: What has been the observation of the duration of the titer following a natural infection?

DOCTOR KENZY: At least six years has been reported. In humans, in some outbreaks it has been ten years or more in some outbreaks I have heard of.

DOCTOR YORK: I might say, in regard to serological tests where you are trying to determine whether the herd is infected or whether the given animal has had a recent infection, I would highly recommend that you read Doctor
Stoener's report given at the professional symposium in Kansas City which has been published in VETERINARY MEDICINE. It is a paper much longer than this report.

It is important to realize that, following infection, the titer is usually much higher than following vaccination, and also that the longer the animal has been infected, the more the titer drops off. Hence, the preliminary screening on the plate test is only to pick out positive serum samples.

To further evaluate that herd, he feels that it is very important to then run dilutions. A positive serum sample of 1:40 is only considered suspicious, and it is only with the next dilution, 1:80 or 1:160 that you can begin to consider it of value.

The titer will be of great assistance in telling you the status of the herd when you are trying to evaluate the situation.

DR. A. H. QUIN [Kansas City, Missouri]: Doctor York stated that the titers on the screen test are not significant unless they exceed a 1:40 agglutination. There are thousands and thousands of blood samples of both cattle and swine submitted for brucella testing, and also requested leptospira tests. In many of these instances I think it is simply the screen test that is run.

If these are titers on the screen test of herds of cattle or swine where there has been no history of trouble, and if they do not exceed 1:40, is the returning of the evidence to the veterinarian as being positive any justification for instituting the expense of vaccination in those herds? Is that capitalized on unduly at present? I would like to ask Doctor York to discuss that briefly.

DOCTOR YORK: I imagine Doctor Kenzy has some thoughts on that question, too.

As I pointed out a few moments ago, the blood test is only an aid and never should be considered a plus or minus answer all by itself. When you have a herd in which you suspect leptospirosis and you can't tell for sure by clinical means, and if you have animals in that herd that are having the disease at the moment, and if those animals are negative to the serological test, and if there are others that have had a similar type of disease 10 days or two weeks previously and are positive to the test, then probably the plate screening without the use of capillary tubes for dilution would be of value.

However, if they don't go to the point of trying to give more than one serum sample from different types of situations in the same herd, then it is important that the dilutions be run in order to evaluate whether this is a titer that could be of immediate significance due to its height, rather than just an indication of past history and that the disease you are seeing now is really something else.

I don't know whether I have made myself clear. You either match it up based on what you are observing clinically, or you have to run dilutions to get a significant titer.

DR. A. H. KILLINGER [Fort Dodge, Iowa]: There is another point about these infected cattle. In checking against other sera types, in the Stoener test an animal that may run a titer of 1:640 against leptospirosis very often
LEPTOSPIROSIS

will react positively with the preliminary screening test against canicola or against ichterohemorrhagica. It is particularly important at that stage that dilutions be run so that the instances of some of these other sera types is not reported as being the cause of the infection.

There is quite a bit of confusion on that point. I think that emphasizes again the importance of running the dilution test on the positive serum.

DR. M. G. FINCHER [Ithaca, New York]: I want to ask Doctor York if the blood should be sent to the laboratory or should be done in the office.

DR. YORK: That might be a twofold affair. The plate antigen appears to be quite suitable for the average practitioner to use in his office only as a screening procedure, and then he can send the serum samples to a laboratory for dilution tests, because I do not believe the average practitioner could run a capillary tube test by himself.

However, if he goes to the trouble of getting a number of serum samples from different types of animals in the same herd, he might get some benefit by using the plate test, but it should not be abused in that regard.

DR. A. H. KILLINGER: Running out these dilutions, it is also possible to run those that Stoener has described. It is possible to run that technique also on a plate.

Some laboratories are running very large series of samples. Stoener feels you can run more samples with the capillary tube test. For only a few is the plate dilution test satisfactory, and some practitioners who have an aptitude for laboratory work are doing that. Others are sending them in to central laboratories for dilution testing.
INFECTIOUS BOVINE ULCERATIVE STOMATITIS

Lafayette, Indiana and Gainesville, Florida

The authors are indebted to Dr. G. M. Neher, Purdue University, for taking the photographs and to Dr. M. P. Jaggi, University of Florida, for taking the electron photomicrographs.

A disease characterized by erosions and ulcerations of the oral mucosa has been encountered in cattle in Indiana. It was called infectious bovine ulcerative stomatitis (9).

The oral erosions and ulcerations that are characteristic of infectious ulcerative stomatitis resembled in certain respects those that occur in a number of other cattle diseases. This disease could be confused with the ulcerative stomatitis of calves described by Gibbons (5), virus diarrhea (1), mucosal disease (10), vesicular stomatitis (2), and so-called mycotic stomatitis (11) which appears to be the same as muzzle disease (6).

CLINICAL

The only clinical signs noted were ulcers and erosions in the mouth, muzzle and anterior part of the nasal cavity, anorexia and marked loss of body weight.

The lesions began as small, irregular, somewhat circular, reddened, pea-sized, superficial erosions. They gradually became larger and deeper until ulcers an inch or more in diameter and three-eighths of an inch deep were formed. They were present on the dorsal and ventral surfaces of the tongue (Fig. 1), lips, buccal mucosa, palate, muzzle, nostril, anterior turbinates, and the skin surrounding the mouth. The skin lesions were perfectly round and most of them were about the size of a quarter. Affected animals often attempted to eat; but hay and concentrates irritated the lesions and caused the animals to protrude their tongues, drop feed already in their mouths, stand with their mouths wide open and shake their heads from side to side, and in other ways exhibit soreness of the mouth. Marked bleeding from the ulcers in the mouths and nasal cavities occurred.

No alteration in body temperature was noted. No hematological changes were detected. The morbidity rate was near 100 percent but no animals died. The course of the disease was two to three weeks in individuals and about six weeks in a herd.

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3 From the Department of Veterinary Science, University of Florida.
No gross lesions were found except the erosions and ulcers in and around the mouth.

Histologically, changes were confined to the epidermis and immediately adjacent parts of the corium. The earliest recognizable lesions consisted of areas of ballooning of the stratum spinosum (Fig. 2). This ballooning appeared to result from vacuolation of the cytoplasm accompanied by pyknosis and karyorrhexis of the nuclei (Fig. 3). The ballooning was responsible for some of the small grossly visible raised areas. Later coalescence of the ballooned cells resulted in the formation of vesicles. This appeared to accomplish the undercutting of the overlying stratum granulosum and stratum corneum until sloughing occurred (Fig. 4). At this point in the development of the lesions, secondary bacterial invasion probably occurred as evidenced by accumulations of inflammatory cells (Fig. 5), e.g., neutrophils, lymphocytes and eosinophils. The next stage appeared to be the development
of a necrotic ulcer extending a short distance into the corium (Fig. 6). Frequently the craters of the ulcers were filled with necrotic tissue debris and inflammatory cells. Inflammatory changes were rarely seen in the corium except in the immediate vicinity of an ulcer. As healing began, granulation tissue developed at the junction of the ulcer and the relatively normal corium (Fig. 7). Healing then proceeded fairly rapidly leaving little or no grossly visible scar. No significant histological changes were noted in any of the other tissues.

No fungi were noted in sections stained with periodic acid-Schiff stain. No bacteria that were considered to be significant were isolated from the lesions.

ETIOLOGY

Infectious ulcerative stomatitis was reproduced experimentally (Figs. 8 and 9) by exposing scarified oral mucosa and muzzle of susceptible calves and adult cows to scrapings from early lesions obtained from naturally occurring cases. The agent was passed serially from animal to animal by swabbing scarified areas of the muzzle or oral mucosa with scrapings from lesions.

Calves inoculated intramuscularly developed slight oral lesions. Scarification of the oral mucosa at the time of intramuscular inoculation did not alter
the nature of these lesions. Inoculation of scarified skin did not result in the formation of skin lesions. Infectious ulcerative stomatitis also spread by contact. The ulcerations were not as pronounced following contact exposure as those induced by local inoculation of scarified lesions. No other clinical signs were noted in any of the experimentally infected animals. No hematological changes were detected.

Selas 02 filtrates of saline suspensions of ground scrapings were infective. The agent remained viable for at least nine months at $-40^\circ C$. Repeated freezing and thawing destroyed its infectivity. The ability of the agent to produce lesions was not altered by incubation for two hours at $37^\circ C$ with penicillin and dihydrostreptomycin so that the final concentrations were 10,000 units and 10 mg. per ml., respectively.

The agent was purified by the method of Gessler (4) and Manson (7) using a total of four fluorocarbon treatments. Fluorocarbon purified suspensions of agent were diluted and examined with the electron microscope. Virus particles were roughly spherical and between 125 and 150 millimicrons in diameter (Fig. 10).

Animals that had recovered from experimental infections were resistant to reexposure by scarification. Calves that had recovered from and were immune to virus diarrhea were susceptible to ulcerative stomatitis.
The horse, pig, sheep, guinea pig (foot pad) and mouse did not develop disease when exposed to virulent agent. Some success was obtained in propagation of this agent in cultures of bovine kidney (Figs. 11 and 12), human liver (Chang) and intestine (Henle) cells. Cytopathogenic effects
were observed at 24 hours which progressed to obliteration of nearly all cells in five days. Serial passage lengthened the time for the appearance of changes and reduced the severity. The effect was absent in the fifth passage. Material passed through Selas 02 filters or purified with fluorocarbon retained its ability to produce these effects. In the one trial in which convalescent serum was used the effects were reduced markedly but not eliminated.

DISCUSSION

Infectious ulcerative stomatitis probably is not a new disease even though a description of this condition could not be found in the veterinary literature. One of the authors (W.R.P.) had previously investigated the occurrence of conditions that were clinically indistinguishable from infectious ulcerative stomatitis in cattle in both Indiana and Wisconsin.

Infectious ulcerative stomatitis would seem to be an important cattle disease for two reasons. First, even though no animals have been known to die from infectious ulcerative stomatitis, this disease causes significant economic loss in a herd from loss of body weight. Secondly, infectious ulcerative stomatitis is similar clinically to two important exotic diseases; foot and mouth disease (3) and rinderpest (8), and could easily be confused with them. Therefore, it is of prime importance to be able to distinguish it from these diseases with a high degree of certainty.
Infectious ulcerative stomatitis appears to be similar in some respects to the ulcerative stomatitis of calves described by Gibbons (5). Gibbons reported that temperatures may reach 105°F., that diarrhea and leucopenia are present and that animals recover in four days to one week. No febrile signs, leucopenia or diarrhea were observed in naturally occurring or experimental cases of infectious ulcerative stomatitis and the course of the latter disease is two to three weeks. Relationship of these diseases must be determined by specific serological procedures.

Clinically the oral erosions and ulcers were identical to those that occur in virus diarrhea (1) and mucosal disease (10). The other clinical signs such as: fever, leucopenia, nasal discharge and diarrhea and the erosions and ulcerations throughout the alimentary canal which are characteristic of virus diarrhea and mucosal disease were not present. The vacuolation of the cytoplasm of the cells of the stratum spinosum was similar to the histological changes that occur in virus diarrhea (1) and mucosal disease (10). Virus diarrhea immune calves were susceptible to experimental infectious ulcerative stomatitis. Although complete cross-protection tests with mucosal disease, virus diarrhea and infectious ulcerative stomatitis have not been conducted, clinical and pathological observations indicate that these diseases are separate entities.

The oral lesions of infectious ulcerative stomatitis resembled to some extent advanced lesions of vesicular stomatitis (2). Gross vesicles or lesions on the feet or udders were never observed in infectious ulcerative stomatitis. Vacuolation begins intercellularly in vesicular stomatitis rather than in the cytoplasm of the cells of the stratum spinosum as it does in infectious ulcerative stomatitis. The horse, pig, sheep, and guinea pig are all susceptible to vesicular stomatitis, but none of these animals could be infected with infectious ulcerative stomatitis. This virus is about two times the size of vesicular stomatitis virus. Infectious ulcerative stomatitis convalescent serum does not neutralize vesicular stomatitis virus in embryonated chicken eggs. It seems likely that infectious ulcerative stomatitis and vesicular stomatitis are separate diseases.

Infectious ulcerative stomatitis did not greatly resemble so-called mycotic stomatitis (11) which appears to be the same as muzzle disease (6). So-called mycotic stomatitis occurs only in the late summer and early fall and is characterized by necrotic ulcerations of the muzzle, oral cavity and in severe cases, the entire alimentary canal. One of the most characteristic clinical signs of this disease is severe lameness caused by laminitis. The skin in the coronary region becomes hyperemic and very sensitive to the touch. Erosions of the udder and the perineal region commonly occur. A marked fetid diarrhea occurs in severe cases. Vigorous efforts have been made in our laboratory to transmit so-called mycotic stomatitis but they have not been successful. The only marked clinical signs in infectious ulcerative stomatitis are ulcers in and around the mouth, anorexia and loss of weight and infectious ulcerative stomatitis can be transmitted readily.
SUMMARY

The chief clinical and pathological characteristics of infectious bovine ulcerative stomatitis are described. Some of the physical and biological properties of the causative agent, a virus, are presented. Infectious ulcerative stomatitis is compared to the ulcerative stomatitis of calves described by Gibbons, virus diarrhea, mucosal disease, vesicular stomatitis, and so-called mycotic stomatitis or muzzle disease.

REFERENCES

REMARKS AT OPENING OF MASTITIS PANEL

M. G. FINCHER*

Ithaca, New York

It is my function and purpose to announce each speaker and no introduction is needed. All of the men on this symposium are well known. I may not have time to attempt a summary of what seem to be important facts or suggestions which each has made. I hope you will write down questions and hand them to me during the procedure or at the end. We may have time for a few quick answers and some frank discussion at the end.

Therefore, I will take the liberty at the beginning of listing a few of the points about bovine mastitis upon which there is some semblance of general agreement among people working with bovine mastitis.

First, any dairyman or veterinarian will agree that it is a complex disease with many known and unknown causes.

Research people throughout the world agree that Streptococcus agalactiae is an important cause. Many would say the important cause. Until owners develop entire herds that are at all times free from this infectious agent we can not accurately state how much mastitis is due to other causes. That is, cows once infected with S. agalactiae may have been damaged, even though the herd is classed as free of this important specific infection.

It is agreed that acute, severe clinical mastitis is largely due to causes other than S. agalactiae. Unfortunately, too little is known about the nature of these other bacterial and environmental causes. All would agree that trauma, including that caused by milking machines, is an important cause.

I think, in addition, that all of us agree that the importance of genetics has not received sufficient attention. Compact udders as opposed to pendulous udders are becoming much more common and should be less susceptible to injury. Many other genetic possibilities deserve study, particularly details regarding the teat end and the ease or difficulty with which infection enters hard milkers, easy milkers or cows whose teat ends are covered with virus warts.

Treatment from day to day in large herds under the supervision of an experienced veterinarian reduces the losses from mastitis, whatever the cause may be. Blindly treating without regard for environmental and infectious causes leaves much to be desired. Treatment alone is not enough to solve the problem. Improper treatment with anything short of sterile equipment and surgical cleanliness causes serious and exotic infections to occur.

Control and prevention of mastitis have not reached the stage where absolute freedom from acute or chronic mastitis can be guaranteed, even with

the complete cooperation of the owner. Yet many owners and veterinarians
are sure that *S. agalactiae* can be easily and quickly eliminated from herds.

After this has been accomplished the problem is greatly reduced. Herds
are freed of *S. agalactiae* by treatment of all infected quarters based on the
use of Murphy's so-called Camp test or by use of some other laboratory pro-
cedure. This is easy in most small herds, but requires repeated cultures and
prompt follow-up treatment in many individual stubborn infections.

Cows with bad fibrotic udders must be kept dry for a year or sent to
slaughter or used as nurse cows regardless of the type of infection.

We will agree that collecting milk samples, running the Camp test and re-
peating this process as required to free herds of *S. agalactiae*, is expensive.

Teaching the owner to prevent acute mastitis is in general pretty well ac-
complished by the time he has freed his herd of *S. agalactiae*. It is the many
exceptions to this rule and our lack of detailed facts that make the problem
seem complex.

We hope each of these eminent speakers will help us to reduce the number
of unknowns at least a little bit today. We hope we may find many other
points upon which there can eventually be general agreement.
THE ECONOMIC IMPORTANCE OF MASTITIS

DR. C. D. VAN HOEWELING

Washington, D. C.

In considering the economic importance of mastitis, let's make some entries in a hypothetical ledger—and look at our future needs, assets and liabilities. Conservative estimates are that we will have a total population of 220 million people by 1975.

At the 1956 level of consumption, we would need more than 155 billion pounds of milk in 1975. That's something like 30 billion pounds more than we produced in 1956—the highest production year on record. Furthermore, nutritionists tell us that we should be drinking more milk. If we increased our average per capita consumption only 10 pounds a year, we would need another two billion pounds a year by 1975.

Our assets include a steadily increasing production per cow. In 1956, this production set a new record—a little over 6,000 pounds per cow for the year. Dairy husbandrymen say that it is possible to maintain the same rate of increase, adding about 100 pounds a year to average production, for the next several years. This will be a vital factor in meeting needs of 1975.

Our assets also include increasing knowledge about breeding, management and nutrition, and better control of diseases and pests.

On the other side of the ledger, let's enter some of the liabilities.

Long-range needs force us to recognize that the dairy cattle population is declining. This is a potential liability. In that case, even with expected increases in production per cow, we would have to halt the current trend or reverse it to give our people in 1975 as much milk per person as we are getting now.

Diseases and pests are continuing liabilities. During the 10-year period, 1942-51, cattle diseases reduced milk production by an estimated six and a half billion pounds a year. That's about one-fifth of the increase needed by 1975. New knowledge and better control may have brought some of these losses down in recent years. How much, we do not know. We do know that mastitis accounts for more milk loss than all other diseases together. It is the most important economic disease of cattle in this country.

Milk losses from mastitis in 1956 are estimated at about 5 billion pounds. That's enough to provide a quart of milk a day for a year for more than six million people. We expect our population to increase by six million people in the next two years.

Milk losses from mastitis of more than $200 million last year represent a conservative estimate figured at four percent of total milk production. Surveys in various states indicate that such losses range from two-plus percent to more than eight percent.
These losses are only part of the story. We do not know how much is lost in lower milk checks for poor quality milk. We also have to consider the cost of replacement when it no longer pays to keep a chronically infected cow in the herd. About three percent of the total dairy cow population has to be replaced annually because of infected udders—at a cost in 1956 of about $34½ million. About three cows out of a thousand die from mastitis. This adds another $8½ million. Therefore, losses total more than $245 million for 1956.

Cost of treatment is another liability. Although we do not have an official estimate of these total costs, one pharmaceutical house estimates that the dairy industry is currently spending $15 to $20 million a year for mastitis preparations of all kinds.

A farmer in Wisconsin reported that it cost him $100 to clear up mastitis in his herd. Another estimate is that milk losses and cost of treatments probably run $300 a year for a 20-cow herd. That's back on the farm—where the farmer can feel it in his pocketbook.

Currently, these liabilities are an economic burden to the industry. However, the burden will be much greater if we fail to reduce these liabilities. Failure to act effectively would make a big difference in our ability to meet the needs of 1975.

There is a bright side to the story. Mastitis can be controlled and some types completely eliminated from a dairy herd by good management, accurate diagnosis and treatment.

A study made in Illinois covered 25 herds, with an average of about 19 cows in each herd. The observation period ran two years. The cows were under the mastitis control program for about one and a half years. Infection went down and production went up.
In these herds the number of cows shedding streptococci in the milk dropped from 37.7 percent to 11.7 percent during the two-year observation period. An increase in milk production occurred almost immediately. During the control program, milk production went up from an average daily yield of 25.9 pounds to 29 pounds per cow. This was more than a three-pound increase in the average daily output per cow for the two-year period. Furthermore, the dairymen received larger milk checks than before, and their returns over feed costs were greater.
Think what it would mean to the dairy producers if all dairymen, through the control of mastitis, could achieve comparable gains of 10 percent in efficiency of production with comparable increases in income and profit.

One study in Massachusetts, limited to five herds numbering 212 cows, indicated even more dramatic gains. After a control program was set up, production per cow increased by 1,929 pounds a year.

In drawing a balance, let's sum up.

Our needs ahead are increasing with a rapidly increasing population and with growing awareness of nutritional needs. Our assets include a steadily increasing milk production per cow, and increasing knowledge on breeding, feeding and handling. Our liabilities include tremendous losses due to diseases, with a loss of around five billion pounds last year to mastitis alone. This is more than one-sixth of the increase required to meet needs in 1975. At 1956 prices, the losses from mastitis represent almost a quarter of a billion dollars of production potential. This is 24 times what the Federal and State Governments spent in 1956 for all animal disease and parasite research work.

That's a high price to pay for a single disease.
THE INVADING ORGANISMS AND THE HOST IN BOVINE MASTITIS

James M. Murphy, V.M.D.*
Ithaca, N. Y.

There is a growing suspicion in the minds of an increasing number of people that the great reliance placed on the antibiotics in the last 10 years—almost to the exclusion of the development of basic knowledge—has been a mistake of the first magnitude. Articles such as the sensational "Has Mastitis Met Its Match?" of a decade ago are giving way to others figuratively, if not actually, entitled "Have We Been Caught With Our Trousers Down?" Sometimes the truth is not pleasant, but pleasant or not, the truth in the present case is that we are caught in just such an awkward position.

Unless you feel that you simply must make things unnecessarily complicated (and it is becoming more and more popular to say that there are 20 or more kinds of mastitis) there is really no need for considering more than four main forms of the disease (Fig. 1). These are mastitis caused by (1) Streptococcus agalactiae, (2) other streptococci, (3) staphylococci, and (4) bacilli, and at least 99 percent of mastitis falls into these groups. Based on figures gathered (and estimates made) over the years in New York State the breakdown is as follows: 50 percent of the cows are not infected with any pathogenic bacteria, 23 percent are infected with S. agalactiae, 13 percent are infected with other streptococci, 13 percent are infected with pathogenic staphylococci, and one percent are infected with bacilli.

Figure 1 also attempts to give some idea of the frequency of symptoms shown by the four forms of mastitis. It should be noted that most infections are in the non-clinical stage at any one time, and thus might be classed as normal when the ordinary barn tests are used. About 24 percent of infected animals are in the mild clinical stage at any one time, and thus would be detected by the careful application of the ordinary barn tests. Finally, only about one percent of infected animals are in the severe clinical stage at any one time, and thus have acute swelling of the gland or are generally ill. It should be indelibly impressed on our minds that all four forms of mastitis can exist in each of the three stages. That is, they can "quiet down" from the clinical stages to the non-clinical stage, or they can "flare-up" from the non-clinical stage to the mild or severe clinical stages. It should be noted further that S. agalactiae rarely causes severe clinical mastitis, whereas some of the staphylococci and the bacilli tend toward the severe clinical rather than the mild clinical.

For more than 10 years, two states in this country have conducted formal mastitis control programs. Connecticut has based theirs on the classical work of Plastridge (1), which demonstrated conclusively that one of the four main

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forms of mastitis—that due to *S. agalactiae*—could be eradicated from herds. New York has based their program primarily on the concepts and beliefs of Udall (2), that the conscientious application of non-specific measures such as sanitation and proper milking machine operation could push down the clinical manifestations of each of the four forms of mastitis. It is needless to say that, while both approaches were formulated before the advent of the antibiotics, each has been enormously benefited by modern treatments.

The Plastridge approach of short-interval testing and treating can eliminate *S. agalactiae* (Fig. 2, area A), and the Udall approach can reduce the clinical manifestations of mastitis (Fig. 2, area B). However, it is perfectly obvious
from the facts of the disease that the elimination of S. agalactiae leaves the other streptococcal, the staphylococcal, and the bacillary forms untouched. On the other hand, efforts to reduce the amount of clinical mastitis that is visible at any one time do little or nothing about the large body of infected individuals from which the clinical manifestations arise (Fig. 2, area C). Area A can be eliminated, and it only remains for a group to decide whether it is worthwhile economically and politically. Area B is typically a veterinary practice area, and obviously could never lead to a complete mastery of the situation. The invasion of area B by farmer-treatment is bringing more and more people to the realization that trying to patch things up when they have reached the clinical stage is essentially getting us nowhere. Area C is the tough area, and the most important one once we realize that we must learn how to stop mastitis before it starts. Extremely little is known about how any form of udder infection gets started, and unless we are much luckier than we have been in the past, we must begin the laborious and costly process of getting the necessary facts.

It was with this in mind that we began our basic studies nine years ago at Cornell. S. agalactiae infection was selected as the best form with which to work because more was known about it and because it was easy to eliminate from the gland by means of penicillin. It was reasoned that, if we could
solve the riddle of this form of infection, the methods and techniques found to be of value would help solve the other forms of infection.

From my work up to 1946 in New Jersey with the age factor and genetic differences between families of cows, it was a fairly good bet that the host part of this host-parasite relationship possessed a resistance that was of major importance (3, 4). As a matter of fact, this was demonstrated experimentally in 1949 by Lancaster and Stuart (5) in England when they showed that younger and previously uninfected cows resisted artificial exposure whereas older and previously infected individuals succumbed quickly to such artificial exposure.

At that time it was considered by such workers as Little (6) that S. agalactiae was sucked upward through the teat canal (Fig. 3) into the teat cavity and that it was here in the teat cavity that the bactericidal or bacteriostatic property of the milk exerted itself to prevent or to allow the bacteria to grow and cause an infection. If this were true, then one should be able to demonstrate great differences in the growth-supporting properties of milk removed

![Diagram of teat cavity and teat canal](image)
from the gland, and one should be able to place small numbers of *S. agalac-
tiae* in the teat cavity with infection occurring only in certain individuals. Experiments designed to test both of these points showed in 1952 and 1953 that neither is true (7, 8, 9).

As a further check on this so-called back-suction hypothesis, it should be true that *S. agalactiae* placed in the outer end of the teat canal would be sucked into the teat cavity. So we designed a swab exposure method which was able to place about five million organisms in the outer three mm. of the canal (10). Then by drawing milk samples through the canal in the conventional manner, and by obtaining milk samples by syringe through the wall of the teat (11), we found that in the vast majority of instances (86 percent) the organisms were just washed out of the canal during the next four or five milkings (12). Thus I think we have adequately disposed of this explanation of the way in which *S. agalactiae* infects a cow.

Fortunately, the swab method of artificial exposure had by this time shown itself to be somewhere close to the natural in that the overwhelming majority of first-lactation cows had proven resistant to such exposure. It was then our very good fortune to obtain a group of six first-calf cows two of which proved to be highly susceptible and two highly resistant to four swab exposures in a three-month period (13). These two contrasting pairs of animals offered the first opportunity to study possible differences in the teat canal in relation to susceptibility. A way was devised by which to measure the length of teat canals without interfering with the experimental exposures (14); and the bacteria-retaining ability of the canals was measured by exposing them while under the protection of penicillin (15). Neither of these was found to be related to susceptibility. Another possible factor, the dilatibility of the teat canal, was studied in these and other animals to see if easy milkers were more susceptible than hard milkers, as was observed by Dodd and Neave (16) in natural infection of the udder. We could find no such relationship (12).

Turning from the fruitlessness of our attempts to correlate physical characteristics of the teat to susceptibility and resistance, we decided to apply mild abuses to the teat canals of resistant first-calf cows in an effort to break their resistance. The abuses were: (1) the repeated insertion part way into the canal of a small, sterile, cotton swab; (2) the repeated application of a milking machine for 10 minutes at 17 inches of vacuum after all the milk had been removed from the glands by normal machine milking; and (3) the removal of the soft keratin lining from the teat canal by means of polyvinyl tubing. Neither the insertion of the sterile swabs, nor the application of excessive vacuum for a prolonged period was able to break the resistance to *S. agalactiae*, but the removal of the soft keratin lining broke the resistance of every teat to which it was applied (17).

This is as far as we have gotten with the limited facilities, manpower, and encouragement we receive. Unfortunately it is still true that no one, anywhere, as yet knows how an udder infection takes place in nature. Anyone who thinks that mastitis as a whole can be truly controlled without such information is, of course, free to go ahead without it, but we would be well-
advised to get fundamental studies started at many institutions. These studies should be well-staffed and have adequate facilities with particular emphasis on the availability of experimental herds of cows. Above all we must be very sure to provide an atmosphere in which basic research can flourish.

REFERENCES


17. Murphy, J. M.: To be published.

DISCUSSION

Doctor Finch: I am sure the representatives of the dairy industry and others who have heard Doctor Murphy's talk will agree that a great deal more money should be poured into the research on bovine mastitis, and that large herds should be made available for basic research. It is very difficult to secure such herds.

If there are any questions at this point, I will call for them now.

Dr. R. J. Schroeder [Los Angeles County]: I didn't quite understand the part about the breaking down of the lining. I wish Doctor Murphy would go over that again.

Did I understand him to say that it was not broken down by milking with a milking machine for 10 minutes after the normal milking period?

Doctor Murphy: I believe your question was whether the application of the milking machine after the udders were milked failed to break down the resistance of the animal. That is correct.

I want you to be sure to understand that this was a specific application of the machine. This does not apply to all machines everywhere, under any circumstances, but we were unable to break the resistance of the heifers by that process of high-vacuum, prolonged milking.

Doctor Finch: I think the question included how you got the keratin lining out of the teat canal.

Doctor Murphy: You might visualize it as merely skin rolled around on itself. As you know, the skin produces a keratin which exfoliates. The skin cannot exfoliate very well because it is rolled around and is growing inward toward itself. Keratin is growing all around it, in toward a central core. It is that sort of keratin lining which, if it is removed very gently by means of a plastic tubing, makes the teat susceptible (100 percent of the time in our work) to the artificial exposure method that we use. It does not necessarily relate to nature directly, but I could take time later on to show you how it might relate to the natural infection.

I don't mean to imply now that it does directly relate to natural infection, except that that is the only way we have been able to break the resistance, namely, through changing the surface of the teat canal by that method.
DOCTOR FINCHER: Are there any other questions? I hear some men saying to themselves that when they insert an open mouthed nozzle in a plastic syringe they are curetting out this keratin, and therefore they are doing what Doctor Murphy is trying to do. I don't believe that is what he wants to imply.

DOCTOR MURPHY: When you use an open end teat tube you will remove some of this material. If you then expose the animal to Streptococcus agalactiae and if she were not then protected by penicillin which you had injected, she might become infected.

Of course, most of the time you are inoculating with antibiotics. So, the question would be, with any organism that could get through at that time, whether it would be held down by the antibiotics you put in. It probably would be, at least long enough for the keratin to grow back again. We don't know how long it takes to grow back, but certainly it is within a week.

DOCTOR FINCHER: We used to have a saying at our various farm meetings that if you wanted to find a herd with really bad mastitis (and this was earlier, when treatment was not as carefully done as it is now), all you had to do was to look for a herd that had a lot of home treatment being done. This may be part of the answer, but it is not appropriate to say that it is the whole answer at this time.
THE POSSIBLE ROLE OF NUTRITION IN MASTITIS

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Reports on the effects of feeding on mastitis generally concern various concentrates, more particularly their overfeeding. This is true of experiments of Hotis and Woodward in 1935 (1), Stang, et al. in 1937 (8), and Moore, et al. in 1942 (3), and the more recent experiences of Reid in 1954 (7). Sometimes higher levels of grain feeding or special components appeared to stimulate mastitis attacks and at other times the opposite was true. Occasionally, the roughage component has been considered as adversely influencing the mastitis situation. For example, Klein in 1922 (2), besides discussing instances involving increased feeding of concentrates, also mentioned a herd problem of fairly sudden onset which was associated with a change from silage to recently harvested hay. Murphy in 1947 (4), summarized the findings of numerous investigators concerning the possible influence of feeds on the disease and it is doubtful if there is anything to be gained by further review of reports made prior to his. In addition, he provided an excellent description of his observations on a large herd over a period of several years.

In the herd studied by Murphy, three extensive outbreaks of mastitis were encountered during a span of six years. Two of these reached their greatest intensity in June, July and August, while the cows were on pasture. The third marked increase in activity of the disease began during the winter and continued for approximately a year with the peak period beginning in April and persisting on into the pasture season, through July. Thus, the peaks included July in all three episodes. Pasture was more plentiful in the two years when outbreaks occurred following turning the cows on pasture than in the intervening years. The possibility of a direct effect of weather or an indirect effect through the feed was discussed by the author.

We have had a number of somewhat similar experiences with herds that indicate the ration, more especially the roughage component, may influence the status of the disease. Neighboring herds grazing on opposite banks of the same river in a midwestern state were both afflicted during the same summer even though neither reported similar problems in previous years. Mastitis attacks in a large West Coast herd were observed to increase when fresh green feed was added to the ration in spring. The number of cows affected varied greatly in different years. For example, in one year, approximately five percent of the animals suffered from mastitis per month prior to using green feed as the sole source of roughage. The month when first on this feeding program the incidence rose to 28.0 percent but a month later dropped again. The following year the rise was from less than 4.0 percent to approximately six percent. In the second year, a preliminary feeding period
during which a quarter to a third of the roughage was composed of green feed extended over several weeks.

One of two relatively comparable groups of eight cows each in the same herd was fed a ration of alfalfa hay, forage crop silage and moderate quantities of grain. The other group received grass hay, mostly timothy of relatively poor quality and more liberal feeding of grain. The observation period was 16 weeks. Clinical evidence of mastitis was manifested by one quarter on each of two cows in the first group and in the second group, five cows had a total of eight quarters between them similarly affected. *Str. agalactiae*, another streptococcus and staphylococci were the associating organisms.

Two comparable groups of 15 cows each were chosen from another herd. Beginning in early May and continuing into the middle of September, one received a forage ration of freshly cut alfalfa and grass mixture while the other received silage made the previous year from similar material (6). Some of the animals in each group received in addition, small to moderate quantities of grain. There was a marked difference in the incidence of mastitis in the two groups. In the first one, there was a total of 27 attacks involving 14 quarters on six cows. Opposed to this, in the second group three cows had 10 attacks half of which were in a single quarter that incurred a serious teat injury. The various organisms recovered by culture from affected quarters were micrococci, both non-hemolytic and slightly so, streptococci, other than *Str. agalactiae*, coliforms, and on one occasion each, a pseudomonas and a fungus. Except for the feedings schedule, all cows were treated the same, handled by the same personnel and milked with the same equipment.

There is a lack of understanding concerning the inconsistencies in manifestations of udder disease, for example outbreaks occur in a herd one year and not in others, in one quarter and not others, and so forth. In some of the above rather extreme problems, a possible connection with the feed might be recognized. But, what about border line situations? It seems plausible that periods of reduced resistance may occur when attacks are more easily incited than at other times. These thoughts and observations have stimulated us to search for possible measures of resistance of animals that would help provide answers to these questions without exposing animals to pathogenic organisms. It seemed logical to examine milk as this is the environment in which the bacteria have to be active in order to produce the disease conditions. The approach has been to check milk samples for their suitability as media or stated differently, to challenge their ability to resist the activity of various mastitis bacteria (5). Capacity to resist acid production has provided the base for measuring the response to *Str. agalactiae*, and the ability to resist change of oxidation-reduction potentials has been used with hemolytic staphylococci and coliforms.

It must be stressed that these methods may not prove to be actual measures of animals’ resistance. Their application has provided, however, an interesting picture because the trends frequently seem to parallel the kinds of variations seen in clinical problems. By observing factors which influence the
test results, additional possible approaches are indicated for future research and application of trial control methods.

Some indication has been obtained in applying these laboratory methods that the feed at times influenced the relationships of milk and mastitic infections.

Experiments on about 50 cows indicated that satisfactory levels of resistance of milk samples to the activity of *Str. agalactiae* were maintained with difficulty when the cows were on rations restricted to hay and grain. This difficulty was seldom experienced when milk production was low, regardless of the quality of the hay fed.

Resistance to the activity of this organism of milk samples from 17 cows on rations of hay, grain, and silage (mostly forage crop silage) was found to be relatively good throughout the winter feeding period, even when milk production was reasonably high. The same was true in some smaller groups fed heavily for shorter periods on this kind of silage, and in others fed moderate quantities of corn silage. An exception to this was a rather severe loss in resistance of milk recorded during the first few weeks after corn silage was added to a hay and grain ration that had been fed continuously for 18 months; however, the former resistance level was recovered and improved upon within a few weeks.

Changes from pasture to rations containing silage were never accompanied by losses in resistance of the milk samples, but losses frequently were noted with changes from winter rations to pasture. One year, in the samples from approximately 40 cows, the resistance loss was more than 30 percent between the weekly test average for the last prepasture month and that for the second month on pasture. The usual trend was for the resistance levels of the milk to improve somewhat after the earlier drop and remain fairly stable during the remainder of the pasture season. In one exception, resistance losses again occurred in September when the pastures had improved due to rains.

Effects of feed on the relationships between milk and other mastitic infections have not been apparent in the test results. However, the average resistance of milk samples to hemolytic staphylococci was lower in the summer than in the winter in the years 1954, 1955, and partly so in 1957. This was not the case in 1956.

Mastitis associated with *Str. agalactiae* apparently has decreased to quite an extent in recent years, more particularly in areas where high quality forage crop silages have been emphasized in dairy herd rations. Should there be some relationship, it is gratifying to know that roughage programs of this type generally are economically advisable.
REFERENCES


MECHANICAL MILKING AND MASTITIS

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Abuses to the udder by mechanical milking are of common occurrence and, unless understood and prevented, mastitis control programs may fail to produce desired results.

As early as 1938, Meigs and co-workers (1) concluded that the changes occurring in the milk of machine milked cows as compared to hand milked cows constitute strong evidence that machine milking may sometimes have an injurious effect on the udder. In a subsequent report (2), these workers stated that the incidence of mastitis, the decrease in milk yield resulting therefrom, and the accompanying changes in characteristics of the milk were in general proportional to the amount of vacuum in the machine and the length of time it was left working on the udder.

Dahlberg (3) reasoned that, since a good hand milker averages about eight minutes per cow, a machine removing milk from the four teats simultaneously should accomplish the complete removal of milk in four minutes. Reduction in machine milking time to four-five minutes per cow was reported to have been accompanied by a fall in total leukocyte count in mixed herd milk and a significant lessening of udder trouble.

Since 1940, dairymen, in general, have been convinced that most cows will milk-out by machine in three to five minutes and that leaving the machine on after milk flow has ceased will increase the incidence of mastitis. Despite general acceptance of faster milking, the milking machine continues to contribute significantly to the causation of mastitis. This suggests that the problem is more complex than simple milking duration and vacuum levels.

Insistence by milk inspectors for more sanitary teat cups and requests by dairymen for teat cups that do not fall from the udder at the end of milking, as well as for machines that are more easily assembled and cleaned, have influenced the design of present day machines. However, while changes in design have tended to satisfy the requirement of convenience, they have not necessarily been in the best interest of the cow insofar as maintenance of udder health is concerned (4, 5, 6).

The milking machine may predispose the udder to mastitis through traumatic injury to the teats and by enhancing exposure of the teats to bacterial pathogens. It is the purpose of this paper to discuss certain basic principles involved in the problem.

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A vacuum is not a force but it intensifies the forces of atmospheric pressure, milk let-down, and blood accumulation in or on the teat. In a complete vacuum, the atmospheric pressure at sea level will raise a column of mercury in a glass tube to a height of 30 inches. This is a pressure of 14.7 pounds per square inch of surface area. A milking machine employing 15 inches of vacuum is operating at a differential pressure of one-half atmosphere and, when the pulsator directs air at atmospheric pressure into the outer chamber of the teat cup, a differential pressure of 7.35 pounds per square inch is suddenly applied to each square inch of surface of the rubber teat cup liner. The amount of this pressure applied to each square inch of teat surface is conditioned by the resilience of the rubber and the degree of tension on the rubber. Use of higher or lower vacuum will increase or decrease proportionately the pressures applied to each square inch of liner surface.

Purposes of the rubber teat cup liner: The conventional teat cup consists of a hollow cylindrical metal shell into which a hollow rubber liner is fitted to establish a sealed space or outer chamber between the liner and metal shell. A small tube is fused to one side of the metal shell to connect the sealed outer chamber with the pulsator which, in turn, alternates partial vacuum with air at atmospheric pressure within the space. An inner chamber is formed when the teat is inserted into the cup and serves to extend the constant vacuum of the mechanical system to the teat.

Milk moves from the teat cistern into the teat cup as a result of differences in pressure within the udder and at the external orifice of the streak canal. The phenomenon of milk let-down forces the milk from the alveoli into the duct system and on into the cistern of the teat. The vacuum intensifies this pressure of the milk causing the teat to balloon and the streak canal to dilate; the milk is then ejected into the teat cup (Fig. 1A). In the absence of milk let-down, the milking machine is unable to produce effective withdrawal of milk from the udder.

The vacuum increases the volume of blood entering but delays its circulation through the teat. Without a positive force to oppose these effects on circulation, the tissues become congested and cyanotic. Air at atmospheric pressure is put to work to massage the tissues and enhance circulation. The pulsator in one phase of its cycle directs air into the outer chamber of the teat cup and immediately the rubber liner moves in upon the teat as a result of the constant vacuum in the inner chamber. Collapse of the liner is most complete below the teat with greatest pressure being applied to the tip of the teat (Fig. 1B).

Potentials for stress or trauma with the conventional teat cup: The diameter of the liner, the resilience of the rubber, and the degree of tension on the rubber are important considerations. The most common liners in use currently are one to one and a half inches in diameter and they have little or no provision for placing tension on the rubber when inserted into the metal
shell. They consist of one piece of rubber which has been molded into the shape desired; for convenience, they will be referred to as large bore, slack liners. Prior to the introduction of this type of liner, it was customary to employ a piece of straight rubber tubing, not greater than three-quarters of an inch in diameter, to form the teat cup. A metal ring was placed at one end to form a mouth and serve to seat the liner in the upper part of the shell. The liner opening formed by the ring was flexible and permitted entrance of teats of different sizes with a minimum of restriction to the flow of milk into the teat. The rubber tubing was pulled through the metal shell under tension and folded over the lower end of the shell to complete the seal. A second piece of rubber tubing with one end expanded was slipped over the lower end of the cup to complete the unit. This type will be called the narrow bore, stretched liner. Molded one-piece liners with narrow bore and a provision for placing the rubber under some tension are available.

Fig. 1. Model of milking machine teat cup in action. A = milking phase. B = massaging phase.
Exposure of the teat to the constant vacuum of the inner chamber of the teat cup causes the teat to balloon and press firmly against the walls of the liner. When a small teat is inserted into a large bore liner, excessive stretching of tissue will take place, which in turn may constitute a stress on the tissues. The degree of ballooning will be dependent upon a number of factors such as: the natural size of the teat, the magnitude of milk let-down pressure within the teat, the level of vacuum, the diameter and resiliency of the liner, and the degree of tension on the rubber. Narrow bore, stretched liners obviously will minimize ballooning of the teat as compared with large bore, slack liners.

It is probable that the large bore liner was designed to ensure rapid filling of the teat with milk under the assumption that this would favor rapid milking as well as to prevent falling of the teat cup. However, experience has demonstrated that it is not necessary to balloon the teat excessively to milk rapidly by machine; and, therefore, the justification for use of large bore liners is open to question, especially when employed in conjunction with a high vacuum level.

When milk let-down decreases near the end of milking, the teat begins to shrink and immediately more tissue is drawn into the teat cup to occupy the space. This is called crawling of the teat cup. The pulling of tissue into the cup tends to close the lumen at the base of the teat. When this occurs, the milk remaining in the udder is trapped and the vacuum extends into the teat cistern resulting in additional shrinkage of the teat. The opposing walls of the cistern at the base of the teat are forced together and rubbed in an up and down motion with each complete pulsation cycle. The narrow bore, stretched liner keeps crawling to a minimum and for this reason milk removal is more complete before the end-point of machine milking is reached and there is less friction on the lining of the teat cistern.

The pressure on the teat produced by the air as it rushes into the outer chamber is significantly greater with a slack liner than with a stretched one. Rubber under tension supplies an opposing force which must be overcome before the liner collapses. This serves to reduce the pressure applied to the teat. Resilience of the rubber also plays a part in countering the air pressure. Resilience is reduced by oxidation of the rubber as a result of absorption of fat. Thus, old liners offer little or no resistance to the differential pressure produced when air enters the outer chamber.

Milk in the teat cavity cushions the pressure wave but when the teat shrinks at the end-point of machine milking the full force of the liner in collapse is applied to the tissues (Fig. 2). The narrow bore, stretched liner tends to confine the vacuum to the end of the teat so that the area of severe compression is limited to the teat end. With the large bore, slack liner, the vacuum extends up the sides of the teat for a considerable distance. In this case, at the end-point of milking, the teat is pushed to one side of the inner chamber as the liner collapses and severe compression occurs from the tip to the base of the teat along one side. The effect of prolonged action of the teat cup after milk flow has ceased can be detected immediately after the cup is re-
moved. With narrow bore, stretched liners, the tip of the teat is hard and ischemic; while with large bore liners, a blanched ridge of compressed tissue extends upward from the tip of the teat along one side.

Significance of vacuum level: The speed of milk removal is proportional to vacuum level. However, the higher the vacuum the greater the pressure applied to the teat by the collapsing liner. A vacuum of 15 inches is required for efficient milking with narrow bore, stretched liners. However, when this level of vacuum is used with large bore liners, the teat cups crawl much earlier in the milking process and the end-point of machine milking is reached when a considerable amount of milk remains in the udder. The tendency will be to leave the machine on in order to obtain a more complete removal of milk but in reality this leads to prolonged action of the teat cup on an essentially empty teat. To minimize early and excessive crawling, when large bore liners are used, the vacuum level should not exceed 10 to 12 inches.

IMPORTANCE OF AIR ADMISSION INTO THE CONSTANT VACUUM SYSTEM

When milk is to be raised above the lowest level to which it has fallen by gravity after leaving the teat, a force is required to push the milk over the incline. In the short-tubed, suspended milking machine, the milk moves into the bucket by gravity and entrance of air into the system is not required.
However, in the claw machine, in which a long milk hose leads directly from the claw to the top of the bucket, or to an overhead pipeline system, the milk must move against gravity. Under such conditions, it is necessary to establish a greater positive pressure behind the milk than in front of it in order to cause the milk to flow in the desired direction.

Fig. 3. Air is required to move milk out of the claw. In the absence of air, the teat may be bathed in milk. At the end-point of machine milking, residual milk in the claw is thrown forcefully against the teat with each opening of the liner in the pulsation cycle. This may enhance spread of infectious mastitis.
Only narrow bore, stretch liners permit the mouth of the liner to remain below the base of the teat on most cows, and as the end-point of milking is approached air leakage into the system is made possible by shrinkage of the teat. Large bore liners permit maximum teat cup crawl, causing the mouth of the liner to seal against the udder proper and little if any air is permitted from this source. If there is no source of air, milk can only be lifted by a positive pressure from milk. This pressure is created within the teat cup liner by the increased volume of milk while the cow is milking and is intensified with each pulsation by the sudden collapse of the teat liner. As the volume of milk diminishes, the collapsing liner of a claw machine will move only that volume of milk displaced by the liner; this is not sufficient to move the milk out of the milk hose. As the liner opens, the volume of space in the liner increases and, in the absence of admission of air, the vacuum level behind the milk suddenly exceeds that of the constant vacuum in front of the milk. This causes the milk to be drawn back into the teat cups where it bathes the teats (Fig. 3). Thus, pathogenic bacteria that have contaminated the claw and milk hose from milking of infected glands may be brought into intimate contact with the teat openings of non-infected glands through the surging of the milk in the claw and teat cups with each pulsation cycle.

An air admission hole in the claw is prerequisite to efficient milking in any mechanical system where milk is moved against gravity. The higher the level to which the milk must be raised the more important is air admission. The elevated pipeline system that does not provide air admission contributes significantly to the causation of mastitis (7).

REMOVING THE MACHINE FROM THE UDDER

It is postulated that the manner of removal of the machine from the udder may contribute to the mastitis problem. At the end of milking, it is common practice for the operator to pinch the milk tube of the teat cup to determine if milk is still flowing. If milk is present in the teat cup, compression of the milk tube may trap the milk in the inner chamber and as the rubber liner collapses against the milk, it is conceivable that milk may be forced into a patent streak canal. On the other hand, if the inner chamber is empty and the milk tube is closed by pinching at the time the liner is opening, the vacuum level within the inner chamber may suddenly be increased by four to five inches. This could conceivably place an additional stress upon the external orifice of the streak canal.

In removing the machine, if the constant vacuum is interrupted but the pulsator is left operating and the milk tubes of the teat cups are constricted by bending the rubber, then perhaps milk trapped in the teat cup may be forced into a patent streak canal by the pumping action of the pulsator on the rubber liner.
RECOMMENDATIONS

1. Employ narrow bore, stretched liners on all conventional milking machines operated at 1.5 inches of vacuum. This applies to both the claw and suspended machines.

2. Discard the liners as soon as there is a noticeable enlargement and loss of resilience.

3. Provide an air admission hole in the claw to prevent surging of milk in the claw and teat cups from the milk hose.

4. Design the teat cup to provide visual milking so that pinching of the milk tube is not necessary in order to determine whether or not milk flow has ceased (Fig. 4).

5. Before removing the milking machine, interrupt the flow of constant vacuum to the teats and simultaneously stop the action of the pulsator on the liner.
MECHANICAL MILKING AND MASTITIS

REFERENCES


DISCUSSION

DOCTOR FINCHER: Gentlemen, I am sure you are very appreciative of Doctor Schalm's excellent description of the milking machine in relation to mastitis. There must be some questions you would like to ask.

VOICE: I was very much interested in Doctor Schalm's remark concerning the cell count. I think quite a lot of the regulatory agencies and industry alike have given it a lot of consideration.

However, there are so many differences of opinion in regard to what constitutes a high cell count, that we are at a loss today as to where to begin. I wonder if Doctor Schalm has any information on that, other than what he has given us.

DOCTOR SCHALM: Yes. It is very important that standards be accepted as to what would constitute the upper limit. I can say that a perfectly normal mammary gland will have less than 100,000 cells per cc. of milk. As the cows approach drying off the counts may go up to 250,000. It is generally said that on an individual cow basis 500,000 cells per cc. of milk indicate that there is trouble.

Concerning bulk milk, I would say that certainly, if the herd was free of mastitis, we should expect less than 500,000. At the present time I would say that if 500,000 cells would be set as the top limit we would be doing very well.

VOICE: Would that be on an entire herd basis?

DOCTOR SCHALM: On bulk milk. It would still mean that there is considerable mastitis present, but it would be an improvement over what we have now.

DOCTOR FINCHER: If there are no other questions, I want to thank you for your attention. I particularly want to thank the members of the panel for doing an excellent job. We thank each of them for answering my request favorably when I asked them to appear on this panel.

Thank you very much. [Applause.]
REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


The limitations of time and space allow only a cursory examination of the status of certain infectious diseases not covered by special committees of this organization. Among the many topics worthy of discussion, this report can include only a few of current special interest and about which relatively new observations may be called to your attention.

MASTITIS

Mastitis continues to be probably the most costly of dairy cattle diseases in this country. The Committee has recognized this by providing for a panel on mastitis in the program. It is our belief that effective management practices are of utmost importance in controlling this disease and that more research is indicated in order to clearly delineate the most important predisposing factors. Some of these needs for emphasis will be apparent in the discussion on the program proper.

While it is conceded that all facts about mastitis are not fully known, there is sufficient undisputed information available which if applied with sound judgement, vigor, persistence and under proper guidance will decrease greatly the economic losses from this disease. At present, it appears that the time is ripe for livestock sanitary officials, veterinary associations, dairy organizations and the Federal and State extension services to join ranks in promoting a great, nation-wide educational campaign for the control of this disease.

SHIPPING FEVER

Of continued interest is shipping fever which has been with us so long and so frequently seen as to almost be accepted as inevitable. It is nevertheless a most important disease and one which merits our close attention.

The work of Carter again calling our attention to the Pasteurella sp., and particularly to Pasteurella hemolytica as an important agent in this disease, has helped to arouse renewed hopes of progress. Clinical infections were produced by exposure of calves to P. hemolytica. The most severe infections were seen in calves that would appear to have had inapparent chronic pneumonia prior to exposure. It was also demonstrated that similar lesions of chronic pneumonia could be produced by means of a filtrable agent.

These findings plus those of Moll and Finlayson in Washington who demonstrated a filtrable agent as a cause of a febrile respiratory disease in cattle
and those of Boiden et al., in California who demonstrated a casual relationship between a virus and pneumonia in sheep, and the clear identification of infectious bovine rhinotracheitis as a separate entity suggest that our knowledge of shipping fever and shipping fever-like conditions may be considerably improved in the not too distant future.

**INFECTIOUS BOVINE RHINOTRACHEITIS**

Extensive reports on this disease have been made at each of the recent meetings of this Association. These reports have indicated clearly that IBR is caused by an infectious agent entirely distinct from that causing virus diarrhea or mucosal disease. The successful propagation of the IBR virus on tissue culture led to useful diagnostic procedures by virus isolation and serum neutralization tests.

Since the report of several controlled field trials of a vaccine last year, rather extensive use has indicated the vaccine to be of practical value in controlling IBR. The longevity of immunity conferred by experimental infection is under current investigation as well as studies to determine the host ranges of the virus, carrier status, and the effects of stress on recovered cattle.

IBR continues to appear in most areas where it has heretofore been reported although not always in such dramatic epizootics. To date the disease has been reported in approximately 30 states, an increase of 10 to 12 since last year.

**VIRUS DIARRHEA AND MUCOSAL DISEASES**

Research is underway in a number of laboratories directed toward clarification of the etiologic relationships between these disease syndromes. It would appear that more than one virus is capable of producing clinical signs of illness similar to virus diarrhea and that mucosal disease can also be reproduced by transfer of an infectious agent from one animal to another. The published evidence suggests that these agents are distinct from that causing rinderpest but continued vigilance is advised until the whole matter is further clarified. It is expected that by this time next year considerable progress will be made toward resolution of the etiology and relationships of these diseases. It is to be hoped that a more definitive report on these viruses and diseases may be given at that time.

**LISTERIOSIS**

Listeriosis continues to pose many puzzling problems to the veterinary profession as well as to public health investigators. The last few years have seen an increasing interest in this disease with the development of new information that is not yet widely appreciated.

The association of *Listeria monocytogenes* with fatal meningoencephalitis in cattle and many other species including man has been known for the past 20 years. However, the importance of this organism as a cause of abortion and possibly of sterility in cattle is an aspect of the disease which deserves far more emphasis in research than it has thus far received. Field cases and
experimental evidence have revealed that this agent is capable of distribution through the tissues of the dam with localization in the uterus. The dam may experience nothing more than an inapparent infection, while the fetus may die in utero and may be aborted or survive only briefly after birth. This syndrome has been recognized with increasing frequency among human infants as well as domestic animal species. In such a situation, attention is focussed upon sources of animal contact by affected humans. The recent isolations of *Listeria monocytogenes* from cows' milk has obvious implications regarding the role of veterinary medicine in the epidemiological aspects of this disease.

However, the search for sources of both human and animal infection should not be limited to domesticated animals. A recent study revealed that multiple animal hosts were involved in a single epizootic. Skunks and raccoons were observed to be dying in an area which soon had several cases of *Listeria* encephalitis among sheep and cattle. A strain of *Listeria monocytogenes* belonging to serotype 4b was isolated from a cow, several sheep, and a skunk in this outbreak. There is reason to believe that wild mammals and birds may serve as reservoirs of *Listeria* infection for both man and his domesticated animals.

Since diagnostic methods for this disease are awkward, the picture of incidence of infection is undoubtedly erroneous. At the present time, the only certain diagnosis is isolation of the organism. This usually means opening the cranium for brain culturing. Following this laborious procedure, bacteriological results may be negative because of peculiarities in the isolation of the agent. Reculturing of tissues after they have been held in the refrigerator for some weeks will often reveal a surprising number of positive cultures which were originally negative. This practice of reculture is cumbersome and time consuming. Basic studies are indicated to understand the nature of this phenomenon and to develop better methods. Serological methods of diagnosis are currently under study in Europe and the United States. However, reliable tests for field application have not been forthcoming.

More studies are needed on the immunological responses of the host to this agent. This is necessary to understand the *Listeria* infection cycle as it takes place in nature and also, to determine the possibilities for active immunization of domestic animals.

**ANTHRAX**

Anthrax this year caused serious losses, especially in Oklahoma and Kansas. This outbreak attracted extensive publicity and affected about 1,749 animals in 581 herds in northeastern Oklahoma and southeastern Kansas.

In Oklahoma the outbreak began during the second week in July. Vaccination started July 21 and thereafter large crews of vaccinators were organized through the cooperation of State and Federal veterinarians and the local practitioners. Individual crew members were able to vaccinate as many as 1,000 to 1,500 animals a day.
Reported cases as of October 29, 1957, involved 457 herds. A total of 886 cattle, 13 horses, 37 sheep, 19 swine, and two of other species died of anthrax.

The noncapsulated vaccine was purchased by the State and offered free of charge to the stock owners. During the early phase of the outbreak, 3,000 doses of #2 spore vaccine was used. Anthrax has not been reported in any of the animals that had received vaccine 10 days previously. Total vaccinations by October 18 involved 3,498 herds, 147,634 cattle, 3,328 horses, 14,247 sheep, 12,281 swine, and 4,859 miscellaneous other animals for a total of 182,349 animals vaccinated.

Strict quarantines were established on movement of animals and animal products. One case of cutaneous anthrax developed in a farmer who cut open an infected cow. He recovered.

In Kansas anthrax was first diagnosed on August 7. Reports up to October 10 indicated that there had been 284 infected herds and 670 cases. General quarantines restricted movement of animals and animal products. Restrictions were also placed for 14 days following vaccination.

Vaccination with noncapsulated vaccine was available at State expense to owners of infected or exposed herds in designated areas; otherwise vaccination was at the owner’s expense. Animals vaccinated totaled 20,821 with a marked reduction of reported cases following initiation of the control program. There were no losses after mid-September. One veterinarian apparently became infected with cutaneous anthrax while conducting necropsies on suspected cases of anthrax.

A lesser outbreak appeared late in August in southeastern Arkansas in an anthrax endemic area already about 80 percent vaccinated. Practicing veterinarians were called to vaccinate the remainder. One veterinarian recovered from cutaneous anthrax on the back of his hand where he had received an insect bite while conducting a necropsy on a cow which apparently did not have anthrax.

Montana experienced its first outbreak in three years in Richland County where 16 animals died on four premises. Old timers living in that area said the last outbreak in that county appeared on the same premises in 1918. On the Idaho-Utah border, 11 cows are reported to have died from anthrax in the first outbreak in 20 years.

The number of deaths reported is identical with the number of cases reported as there was no reliable way to determine whether or not anthrax was involved in the treated cases that recovered. The disease picture was complicated by blackleg, malignant edema, leptospirosis, and anaplasmosis; and the cases reported are only those in which regulatory officials were satisfied that anthrax was the disease involved.

These outbreaks were in keeping with general concepts concerning anthrax. They appeared late in the summer and were preceded by spring floods in Kansas and Arkansas. Flies were numerous. Successful treatment was reported in cattle by use of large doses of penicillin during the early febrile stage. Vaccination was believed to be very effective in curbing these outbreaks.
The Committee is indebted to Drs. F. J. Mulhern and J. L. Hourrigan of the ARS for their invaluable assistance in furnishing information on the anthrax outbreak.

**GENITAL DISEASES**

The widespread practice of artificial insemination not only calls our attention to the heavy economic losses due to infertility and sterility but also provides opportunity both for better control of genital diseases and more rapid spread of genital diseases. As control and eradication of brucellosis progresses, increasing attention must be given to other genital infections.

Newer techniques for handling semen must be evaluated, not only from the standpoint of preserving the well-being of the spermatozoa but also from the standpoint of disease control. This is particularly illustrated by the problem of failure to control vibriosis by addition of antibiotics to extenders used in preserving semen by freezing.

In view of the demonstrated possibility of wide dissemination of diseases such as vibriosis and trichomoniasis by artificial insemination, consideration should be given to the possibility of requiring periodic health examinations on all bulls used in public artificial insemination programs. Genetic defects such as dwarfism, prolonged gestation, and epitheliogenesis imperfecta, should receive consideration as well as the infectious causes of disease. Great care is recommended in making diagnoses of reproductive diseases. It seems probable that not all infective causes are known or commonly recognized. Kendrick, McKercher, and Saito recently reported on a virus causing vaginitis and temporary infertility in California herds. Howarth et al. has reported on an epizootic form of abortion in California which shows evidence of being caused by an infectious agent. All efforts to demonstrate the etiology of this serious problem have been unsuccessful. As mentioned elsewhere the possibility of listeriosis must be kept in mind in investigations of reproductive problems. These constitute several among other examples of disease affecting reproduction which might be cited.

The livestock industry is deserving of our best efforts to clearly delineate and effectively control diseases of reproduction.

**JOHNE’S DISEASE**

Johne’s disease is reported from many states to be causing an increasing problem. Incidence appears to be on the increase and it is a matter of grave concern to affected livestock men, practicing veterinarians, and regulatory personnel. There are repeated incidents of introduction of Johne’s disease into herds and flocks by purchase of animals which in some instances have come across state lines. Diagnosis in individual animals in infected herds is uncertain. Reliable programs for control and elimination of the infection have not been established. It is recommended that Johne’s disease receive the emphatic attention of research workers and regulatory officials. It is suggested also that the United States Livestock Sanitary Association consider the advisability of appointing a special committee on Johne’s disease.
TUBERCULOSIS ERADICATION—VETERINARIANS LEAD THE WAY

J. ARTHUR MYERS, M.D.

Minneapolis, Minnesota

I shall always consider the invitations to participate in the programs of your organization on December 4, 1930; December 3, 1941; December 4, 1946; and today as truly high honors.

Although during the last decade of the 19th century, the veterinary profession proved incontrovertibly that a tuberculin reaction means the presence of tuberculous lesions, it was not until 1908 to 1912 that Ghon working in St. Anne’s Hospital in Vienna confirmed this finding in human beings. He did meticulous post mortem examinations on 184 bodies of children who had no evidence whatsoever of tuberculosis except the tuberculin reaction before death. The only one in which he failed to find lesions was not completely examined. The tremendous post mortem experience of veterinarians since 1916 should leave no doubt in the mind of any person that the characteristic tuberculin reaction indicates the presence of lesions and living tubercle bacilli. Despite this fact most physicians in human medicine until recently clung to the obsolete belief that there is a sharp dividing line between infection and disease. It has been well established that within an hour after bacilli invade, lesions are beginning to form and by the time sensitivity can be elicited by the tuberculin reaction, they are well established. Regardless of their minuteness in many cases at this time, they are unmistakable areas of tuberculosis on microscopic examination and are harboring tubercle bacilli. The only difference between the disease in this stage and that from which the animal or person has died is one of extent. The microscopic lesions are responsible for the subsequent gross incapacitating and killing ones. Such facts so firmly established more than 40 years ago, are only now being accepted by a minority of physicians in human medicine, while the majority employ the tuberculin test little or not at all.

Recently confidence in the specificity and accuracy of the tuberculin test has been shaken by the “no-visible-lesion” tuberculin reactor. Although this is a better term than the former one, namely, “no-lesion” reactor, it still seems inadequate. No-visible-lesion reactor was recommended and adopted by those who believe that lesions in some tuberculin reactors are not seen because most post mortem examinations were not done in sufficient detail. They are of the opinion that if the large bovine carcass could be examined as meticulously as Ghon did bodies of children, lesions would practically always be found. Recently this belief was partially supported when Nassel, Germany, reported pathological and bacteriological findings on 1,000 tuberculin reacting cattle in which no tuberculous lesion was found at the usual post mortem examination. The infection was found in 50.2 percent, of which 86.6 percent detailed gross examination revealed the lesions and 13.4
percent culture or animal inoculation was necessary. Of the lesions found 36.5 percent were in the lungs, 12.1 percent in the mesenteric lymph nodes, and 1.4 percent in the udder or its lymph nodes. Thus one-half of the problem of no-visible-lesion reactors was explained. The bovine carcass is large and lesions may appear in areas remote from their usual sites, therefore one wonders if the remainder of this problem would not be largely solved by more extensive examinations. In any event, until this is done, the specificity and accuracy of the typical tuberculin reactions should not be too seriously questioned.

In recent years mild sensitivity, that is reactions only to larger doses of tuberculin, have been designated non-specific and it has been suggested that they may be due to such organisms as acid-fast saphrocytes. Unfortunately this has not been documented by actual recovery of such organisms and therefore many physicians are hesitant to regard mild reactions to tuberculin as non-specific until more evidence is available. This attitude is largely because sensitivity resulting from unmistakable human or bovine type of bacilli varies considerably in degree at different times in the course of the disease. Furthermore, many cases are on record of persons who reacted only to the larger dose of tuberculin, while at necropsy or pulmonary resection, unmistakable tuberculosis was present and the recovered bacilli were of the virulent mammalia type.

Inasmuch as each of the pathogenic types of tubercle bacilli cause clinical disease in two or more species and each one attacks humans, eradication of this disease requires the destruction of all three types. This necessitates unified efforts by veterinarians, physicians in human medicine, and their allies. The bovine type of tubercle bacillus is the most destructive form in any community inasmuch as it produces just as serious disease in human as in cattle tissues. Moreover, it attacks other domestic animals and pets. In West Germany, last year, it was estimated that 10 percent of the tuberculosis among people was of the bovine type. In Hungary 4.4 percent of people with pulmonary disease and 36 percent with lesions in other organs had the bovine type of tuberculosis. In England until recently, this type was responsible for 50 percent or more of the cases of tuberculosis of the skin and peripheral lymph nodes, 25 percent of the acute fatal forms of disease, such as meningitis and miliary tuberculosis, 20 percent of lesions of the genito-urinary organs and the skeletal system. In some parts of the world it still causes from one to six percent of the pulmonary tuberculosis.

In nations where the campaign against tuberculosis in animals has been waged in the same manner as in the United States, large blocks of tuberculosis in people have disappeared because infection of people by cattle was brought to a halt. For example, in the State of Minnesota when a hospital for crippled children was provided near the end of the 19th century and for some time thereafter, from 50 to 75 percent of the children admitted had tuberculosis of bones and joints. In 1955 the admissions with this disease constituted 0.9 percent. When the long-time medical director of the institution was asked about this changed situation, he said that it became noticeable
soon after pasteurization was extensively employed, but more so as the eradication program among the cattle herds proceeded and also as the disease was better controlled among people. Apparently, it has been unusual for more than 20 percent of the clinical cases of tuberculosis of bones and joints to be caused by the bovine type of tubercle bacilli, but this is a large block which has been practically eliminated in this country. When one realizes that clinical disease of the skin, peripheral lymph nodes, genito-urinary organs, meningitis, miliary disease and pulmonary lesions as well as those of bones and joints formerly caused by the bovine type of bacillus have nearly vanished, one appreciates the tremendous role veterinarians have played in controlling tuberculosis among people.

The necessity of destroying all types of tubercle bacilli is emphasized by the ever-increasing frequency of reports of cattle becoming infected from people. Although the subject has not been carefully studied, it seems probable that a considerable number of people harboring the bovine type of tubercle bacilli which they acquired when the disease was so prevalent among cattle, may transmit these organisms to tuberculosis-free animals where they will cause serious disease. However, the greater likelihood probably is that of people transmitting human type of tubercle bacilli to cattle. While this type usually does not result in progressive disease in bovines, it does produce nodules and sensitizes the tissues to tuberculoprotein. Animals so infected become tuberculin reactors and must be slaughtered inasmuch as there is no method of differentiating between the bovine and human types of infection by the tuberculin reaction.

In fact, periodic testing of cattle with tuberculin in countries where the incidence of reactors is small has become a well-recognized method of finding contagious cases of tuberculosis in people.

Obviously the problem of tuberculosis in animals, particularly cattle, swine, dogs, etc., will not be solved until we eradicate tuberculosis from people.

There are only slightly more than two dozen well documented cases of progressive tuberculosis in people caused by the avian type of tubercle bacilli. This condition may not be as rare as was formerly believed because typing of tubercle bacilli from human cases has been little practiced. Such cases may transmit bacilli especially to fowl and swine where they cause considerable destruction.

We must repeat and repeat and repeat the well established fact that people contract tuberculosis from animals and animals contract the disease from people. Therefore, relentless warfare must be conducted against all pathogenic types of tubercle bacilli.

When this century began, the expectation of life at birth in the United States was less than 50 years. By 1955 this had increased to 73.6 years for white women and 67.3 years for white men making an average of 69.5 years. This increase in life expectancy has complicated the tuberculosis problem.

The people now living of 40 years or older had little protection against tubercle bacilli when they were infants and young adults. It is probable that infections occurred in the vast majority by the age of 18 to 20 years just
as is still true in some parts of the world. It was this generation, like all that had preceded it, that contributed a tremendous number of sick people and high mortality. In fact, it was for those persons in the latter part of the 19th century and the early part of the 20th century that sanatoriums were built. These institutions were filled to capacity and large numbers of tuberculous persons could not be admitted because of lack of space. It was this generation in which mortality rates soared. It is the same generation now, people of 40 or more years, who contribute the great majority of clinical cases and deaths. It is this generation in which the highest percentage of tuberculin reactors exists.

Among people born during the last few decades, there is a tremendous contrast in the tuberculosis situation. The effective control of tuberculosis among the cattle herds, isolation of human cases in institutions, and disseminating information about the contagiousness of tuberculosis everywhere has resulted in a marked reduction in the infection attack, morbidity, and mortality rates among these younger people. Instead of infection being well nigh universal as it was in previous generations, it is well under 10 percent among persons of college and university age in most places, and in the neighborhood of two or three percent of children of grade school age with sizable areas where no child has been infected. The large number of teenage girls and boys and those in their early twenties who were admitted to sanatoriums a few decades ago is now contrasted with the sparsity of persons in this age period now in such institutions.

Along with the marked reduction in infection and morbidity rates corresponding decreases in mortality have occurred. In 1942 in the United States 2,702 children from birth to 14 years died from tuberculosis whereas in 1955 only 370 and in 1956 only 290. Among persons from birth to 24 years between 1942 and 1946, the annual number of deaths was over 9,000, whereas in 1955 this number had been reduced to 780 and in 1956 to 600. In Minnesota in 1956 only two persons under the age of 25 died from this disease. In New Hampshire in 1955 no death from tuberculosis occurred under the age of 35 years.

Although county-wide tuberculin testing has not yet been done on a large scale anywhere in this country, on the basis of what has been done, it is probably quite accurately estimated that approximately one-third of our citizens are harboring tubercle bacilli. This is in sharp contrast to the 0.15 percent of infections among the 95 million cattle of this country.

The magnitude of the present and future tuberculosis problem among people is evident when it is realized that billions of tubercle bacilli exist in each of the approximately 56 million infected individuals. The seriousness of this situation becomes apparent with the fact that a large crop of clinical tuberculosis must be harvested annually among these individuals. Even if only five percent of these persons have their disease evolve to clinical proportions, the future problem is staggering. On the basis of past observation there is good evidence that this will occur in far more than five percent.
On the other hand, the problem is facilitated somewhat in that the vast majority of reactors are among persons in the upper age bracket and that all can be found by the tuberculin test. However, this test cannot be limited to the older individuals for although the number of reactors is small in the earlier decades of life, those who are infected must be found. The problem is also facilitated in that methods are now available which are capable of detecting those with contagious disease promptly and those in whom clinical disease is destined to develop subsequently while it is in the presymptom and noncontagious stage. Furthermore, there are adequate sanatorium beds to provide for contagious cases and there is excellent treatment to prevent those whose lesions are found in the presymptom stage from falling ill and becoming contagious.

We have reached the turning point in our tuberculosis eradication campaign among people. Most time, funds, and effort in the past have been spent in attempting to repair the damage done by the tubercle bacillus. There is still a considerable amount of this kind of work to be done. However, our major effort can now be directed toward eradication of the tubercle bacillus itself and thus preventing damage.

This is one of the most crucial periods in the history of the fight against this disease as a tremendous amount of information must be disseminated to the people of the country concerning necessary changes in the program and emphasizing the vast amount of work that remains to be done. With the marked decrease in tuberculosis morbidity and mortality, a complacent attitude has evolved. All that has really happened is that the first two goals of the tuberculosis campaign are being approached, namely reduction of illness and death.

The 56 million people harboring tubercle bacilli, most of whom now appear well, but all of whom are potential cases of clinical and contagious disease have not been brought adequately to the attention of the public.

This complacency has been accentuated by some tuberculosis associations having announced that they are entering other fields leaving the impression that there is no longer enough work in tuberculosis to justify their existence. It has also been accentuated by organizations and individuals promoting the idea that it is possible to immunize against tuberculosis with the same effectiveness as in smallpox.

I am embarrassed to even mention the phrase “immunity in tuberculosis” in the presence of this audience, and to those who may read this manuscript, because so long ago veterinarians of this country proved incontrovertibly that attempts to immunize against tuberculosis are futile. With all the evidence that was adduced, abandonment of all such efforts should have been universal. However, there are still those who have hoped against hope, and even now are seriously retarding the eradication of tuberculosis among animals in some parts of the world.

Even though the International Office of Epizootic with headquarters in Paris and the World Health Organization have recognized the incompatibility of the use of BCG in animals and have recommended that control be based
upon the use of the tuberculin test, there are still those manufacturing and distributing this substance, who are making exaggerated claims for its efficacy without the slightest supporting evidence. Wherever dependence has been placed on BCG to solve the tuberculosis problem among cattle, a sad situation exists today as compared to places where fundamental methods have been employed.

In human tuberculosis attempts have been made to produce immunity artificially for approximately 75 years. A large number of preparations have been produced and recommended. None has passed the controversial stage. There are approximately one-half dozen so-called “vaccines” now being highly recommended in various parts of the world, and others are in the offing. This despite the fact that it was learned in the 1880’s that such efforts are futile because an attack of tuberculosis, either mild or severe, does not result in immunity.

At present, BCG is in the limelight. It is said to have been administered to 150 million persons largely through the World Health Organization. The literature abounds with articles declaring its efficacy and safety, but no article yet published holds up under careful scrutiny. In many articles “successful vaccination” appears in titles, but on reading one finds that successful refers only to converting individuals from tuberculin non-reactors to reactors.

Every place that BCG has been employed, other factors have been in operation, making it impossible to truthfully assign credit for improved conditions to BCG. For example, in one nation a good tuberculosis control program is undertaken and BCG is included; in another nation, or similar area, the same fundamental program is in operation but BCG is not used; the same good results are reported in each nation; therefore, despite the exorbitant claims for the efficacy of BCG in the nation where it was used, there is no evidence that it played any role whatsoever. However, these exorbitant claims for it get into both professional and lay literature, and may be accepted by persons who make no investigation of facts. Such acceptances have been and still are a serious deterrent to sound tuberculosis work, not only because something valueless is recommended and used, but also because of the complacency that results in the minds of those who believe that people can be immunized against tuberculosis.

The statement so often repeated that BCG is safe has been completely discredited yet it continues to be made. The number of clinical lesions that develop at the site of administration and in regional lymph nodes, often requiring treatment including surgery, is frightening; but even more so is the fact that recently BCG has been found to produce clinical disease in various organs, including the eye and resulting in blindness, kidneys, bones, joints, etc. Several deaths from BCG have also been reported.

Bacteriologic studies have been made of cultures called BCG from several places. They have shown that no two cultures are alike and that each one contains multiple bacterial forms instead of the single organism which Calmette and Guérin described. Moreover, some of the organisms in the so-
called BCG cultures are definitely invasive for animal tissue. Apparently, it is this change that has occurred in cultures that is responsible for the fact that BCG produces progressive and often killing disease in mice on deficient diet, silicotic guinea pigs, normal golden hamsters and ground squirrels, as well as the lesions and deaths in people above cited.

I have spent some time on this subject because it seriously threatens the fundamental program which has brought tuberculosis to a lower level in the United States than that found in any other major nation population-wise in the world. Moreover, it has brought the disease to a lower level in many states than now obtains in smaller nations with similar populations.

If this campaign to use BCG extensively were to succeed, it would nullify the master key—the tuberculin test—which unlocks all doors leading to the eradication goal. To destroy this test would blight all present hope of ultimate eradication of the tubercle bacillus.

Tuberculosis in man is no different from that in cattle. In achieving man’s greatest victory over tuberculosis, veterinarians taught physicians in human medicine many facts; indeed, all that is necessary to eradicate the tubercle bacillus.

Veterinarians have led the way so effectively that their advice and support is greatly needed in solving the remaining tuberculosis problem among people. Participation of veterinarians in this campaign has already been practiced effectively on far too small a scale. The know-how and enthusiasm of the veterinary profession in launching an all-out campaign and perpetuating it is desperately needed and could hasten the attainment of the eradication goal. No such campaign has ever been launched against the tubercle bacillus per se among people. We have been satisfied with small programs here and there. We have emphasized the cost of tuberculosis to the nation, but an all-out nationwide attack against the tubercle bacillus awaits an understanding leader who might well be a veterinarian. Only a few weeks ago in this very city, a tuberculosis organization could not see its way clear to enter a campaign that would ensure much greater success because it “would tax our present manpower shortage by overstimulating the program.” Thus a tremendous opportunity was lost. Stimulating the citizenry of all areas to become active in the campaign against tuberculosis should be an important objective of every tuberculosis organization. With increased interest, the people become aware of the seriousness of the present and future problem and are willing to see that funds are provided for adequate manpower and everything else that is necessary to solve the problem. It has been estimated that tuberculosis is costing three-quarters of a billion dollars in this country annually. Unquestionably, this is an underestimation yet no one has had the ability or the courage to seek funds to cope with such a problem.

When the veterinary profession and its allies visualized the magnitude of the tuberculosis problem among animals, funds consistent with the size of the problem were sought and obtained. The American citizenry will respond just as readily and probably more so when it is informed of the serious threat tuberculosis now holds over the future of the nation.
It has long been recommended that no Board of Health, national, state, or otherwise operate without at least one veterinary member. However, as far as I have been able to ascertain, this has not been followed to any considerable degree. Wherever this has been practiced, as in Columbus, Ohio, it has been found exceedingly valuable.

Likewise, every tuberculosis organization, national, state, and local should have one or more veterinarians on boards of directors and executive committees. Their memberships should include every veterinary physician in the areas they serve. In the State of Illinois, the importance of the veterinarian has been recognized and emphasized. Dr. Paul S. Dodd, County Veterinarian at Danville, has served the Illinois Tuberculosis Association in numerous capacities and was recently president of that organization.

Our country now is greatly in need of the information and help the United States Livestock Sanitary Association and all veterinary organizations have to offer. It would be of tremendous help in the all-out campaign to eradicate pathogenic types of tubercle bacilli, if articles written by veterinarians, livestock producers, etc., would begin and continue to appear in veterinary, other medical journals, in magazines and newspapers explaining why you abandoned so-called immunizing agents, what you have accomplished, the present status of tuberculosis, and what is necessary for the future. These facts should be repeated over and over for there are those who have forgotten and there are many who have not previously heard them. To overcome the present complacency and to keep the public convinced that the most difficult part of the tuberculosis eradication campaign lies ahead is our problem. It is a part of our national defense. A statement by A. G. Carlson, D.V.M., Mayo Foundation, “An animal that reacts positively to the tuberculin test is properly considered as a dangerous individual . . . in spite of the great advances in control, there is a constant potential hazard as long as only a few infested animals exist,” should be repeated over and over. It applies to people the same as to cattle. Once this fact becomes established in the public mind, complacency will be overcome. Until it is, we are in great danger of losing all that has been accomplished. The 0.15 percent of the animals now infected in this country, if not found periodically and dealt with accordingly, could disseminate their tubercle bacilli so the disease would revert to its former proportion. To allow this to happen would be the worst possible economy. If this is to be avoided, the present program of periodic retesting of the cattle of this country must be continued.

Among people one in three is harboring tubercle bacilli and it is easy to see how, if these individuals are not found and prevented from becoming contagious, it would be only a brief time until the tuberculosis situation would be as serious as it was at the beginning of this century.

This is not just the problem of any one group, it is not even just the problem of professional workers, it is the problem of every American citizen.
STATUS OF FEDERAL-STATE COOPERATIVE
TUBERCULOSIS ERADICATION

A. F. R ANNEY

Remember the World Series game a few years back, when the Yankees were ahead one to nothing and had a no-hitter going for them? Then in the last half of the ninth, with two men out, Cookie Lavagetto hit a double that won the game for the Dodgers. That was something to see and be a part of, and I've always felt a little sorry for the spectators who got up and left, at the start of that last half inning thinking the game was all over.

You know, it's bad enough to see the spectators leave a game before the last man is out, but when the players themselves start leaving—well, then it's time for somebody to take another look at the game.

That is exactly what I suggest we do today with the tuberculosis eradication program. The fight against bovine tuberculosis is not over until the last source of infection is wiped out—but some of the players have already started to leave the field. So, it's time to take another look at the whole idea of the game.

That idea is to see tuberculosis eradicated, not quit because we think we have a comfortable lead.

In the last few years, the reports to this Association on the status of our cooperative tuberculosis eradication program have consistently called attention to the progress we've made in reducing the incidence of the disease to its present low level. Now this makes excellent historical background—fine statistics on runs batted in and so forth. But the past is past, and reports of past progress are of little help to us in actually finishing the job—especially if they impress us so much that we become complacent and satisfied with what we've already done.

The plain truth is that bovine tuberculosis is still with us, and we haven't won the fight or done our job till we've mopped up the last traces of it.

Our past victories should serve simply to show us that we can win the final round. We can eliminate tuberculosis. We have but to revitalize our efforts, "dig in and stamp it out," rather than rest on the laurels of past accomplishments and allow control measures to be substituted for eradication.

It is unfortunate, indeed, that severe outbreaks that practically eliminate some of our valuable herds seem inevitable before we are awakened to the necessity for more concentrated action.

It is high time that this organization, the livestock industry, and the veterinary profession cast aside the prevailing lackadaisical attitude toward this

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1 Dr. A. F. Ranney, Chief, Tuberculosis Eradication Section, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
disease and take another look at the goal of our cooperative project: tuberculosis eradication. Take a look, too, at some of the things that are delaying the attainment of that goal.

(Fig. 1) shows where we have found the majority of the tuberculin reactors since the eradication program began 40 years ago. Of the more than four million reactors found so far, 69 percent have come from nine states and 24 percent from 13 other states—which makes a total of 93 percent from 22 states, while only seven percent of the reactors come from the remaining group of 26 states.

The same chart carries the figures on location of reactors for the past three years. In the same nine states, we've found 75 percent of the total reactors in the United States; an additional 11 percent came from the 13 states—making a total of 86 percent of recent tuberculin reactors in the same 22 states, and only 14 percent from the remaining 26 states.

(Fig. 2) shows the total cattle population in the various areas. In the nine-state area where 75 percent of the country's reactors were found in the past three years, only 31 percent of our national cattle population is located. The 13 states where 11 percent of the reactors were found have 18 percent of all our cattle and in the other 26 states where only 14 percent of the reactors were discovered, you will find over half the cattle in the country.
Certainly we must fight tuberculosis where and when we find it regardless of the incidence of the disease in the area. These figures also point to the general locations where we must concentrate our efforts to eliminate bovine tuberculosis from this country.

A careful review of the funds available for tuberculosis eradication suggests that in several states where relatively few reactors are being found, Federal funds will of necessity be reduced in order to support a more intensified program in other states, where a larger number of infected animals are presently being detected.

That's one new shot-in-the-arm we hope to give the tuberculosis eradication project. Then we have the fundamental procedures for disease eradication; and if these procedures are going unused as far as tuberculosis is concerned, it's time to dust them off and put them to work. I have these five points that must be given special attention:

1. First, we must reaffirm our confidence in the tuberculin test, which when properly applied and interpreted is recognized as one of the most dependable disease detection tests we have. All too frequently, undue concern is expressed because some animals that react to the tuberculin test do not show up with gross lesions of tuberculosis on post-mortem inspection. The best scientific evidence and observations of millions of carcasses clearly show that the tuberculin test is more reliable than the customary post-mortem examination as a means of detecting tuberculosis.
During the past year, a set of 60 color slides was distributed to each of our Animal Disease Eradication Division field offices and veterinary colleges. These slides were developed for use in reacquainting veterinarians and acquainting veterinary students with the proper technique and procedures for applying and interpreting tuberculin tests. The slides also provide additional educational information. A series of leaflets—"TB Topics"—is also being distributed through the field stations to veterinarians who may be called upon to apply tuberculin tests. We hope these aids and others to be developed will help in redirecting attention to the approved uniform testing procedures.

2. My second point is this: We must give close attention to the proper quarantine of infected herds, and make certain that no known infected or known exposed animal is the cause of spreading the disease.

You can see how important it is to give careful attention to quarantined herds when you realize that only about four percent of the total tests during a year are made in quarantined herds, and yet that's where we find more than one-fourth of all our reactors.

3. My third point: You can't get along without cleaning and disinfection in tuberculosis. We must assure ourselves that all premises that have harbored tuberculous animals are properly cleaned and disinfected under vigilant supervision.

4. The fourth item I'll mention concerns an area that needs some real tightening up. I guess in baseball lingo you'd call it "scouting" but we know it as epizootiological investigation. We must work at this carefully, thoroughly, and vigorously. We have had tremendous success in locating many centers of infection as a result of tracing procedures, and so it is especially difficult to understand the seeming lack of interest by some individuals in this phase of the project. They apparently take for granted that periodic testing alone is sufficient. Neglect in making every reasonable effort to trace the origin of known-infected animals, or to determine the health status of herds that harbor animals previously exposed in other herds, just cannot be justified:

We must trace to their herds of origin all animals that show lesions of tuberculosis on regular kill. We must determine the origin of animals that react to tuberculin tests; and we must follow up on exposed animals removed from infected herds. In each case, we must apply the appropriate tests to the herds that contain, or that may have harbored, known tuberculous animals or those exposed to infection.

These tracing procedures have had relatively little attention in connection with tuberculosis eradication until recent years, but they are proving extremely valuable in locating infected herds that might not be detected until an area test may be applied some time in the future.

Our tracing procedures sorely need a system that will provide for adequate identification of individual animals and complete records covering their movements. Such information would be most helpful in our work, and we hope that more consideration will be given to this aspect of the program.
A little while ago, I said that our epizootiological investigations must be thorough, and they must be. They must be carried through to include possible association with humans, poultry, and classes of livestock other than cattle that may be infected with tuberculosis of any type.

During the past year, a relatively large number of bovine carcasses inspected on regular kill in one slaughter plant were reported with tuberculous lesions. About 70 percent of these reports have shown that only mesenteric lesions were found. Investigations as to the origin of the animals that had these lesions, as well as limited laboratory studies, suggest that tuberculosis of the avian type may be associated with some of the cases. Complete laboratory tests have not yet been made to determine conclusively the type (or types) of tuberculosis that may be involved.

We anticipate better laboratory facilities in the near future to help in our diagnostic and investigational work on tuberculosis. These new facilities will be a tremendous asset to the overall program.

It is generally agreed that more effort should be directed toward the eradication of tuberculosis in poultry and swine—to help reduce to some degree tuberculosis in cattle. Federal meat inspection records show that three percent of swine slaughtered are retained due to tuberculosis. If slaughter swine could be adequately identified so that those showing lesions could routinely be identified with the farms of origin, we could accomplish a great deal with a minimum of effort and expense in locating tuberculosis in cattle, swine, and poultry on these farms. Possibilities of obtaining help through the use of a tattoo system, presently in use to identify certain lots of market swine, are being investigated.

5. Finally, we must put out the kind of effort in our tuberculosis eradication work that will gain the most with the least expenditure of funds. We must give careful consideration to how often complete area tests are necessary in certain localities, and we must be judicious in selecting herds in other localities where we may be able to achieve eradication without periodic testing of all cattle. I have already mentioned the redistribution of funds to pursue a more concentrated program in those states where most of the infected animals are now being found.

You might call all this the "coach's pep talk" the "give-em-heck-and-go-out-there-and-win" approach. But let me say this in closing. A coach may have a good team, but he must consider the possibility of losing. We may have a low incidence of bovine tuberculosis in this country, but all of us familiar with the program are constantly faced with the fact that many dangerous reservoirs of infection exist to menace our livestock. We've been sliding along with a low incidence for a good many years. Now is the time to revitalize our efforts and actually eradicate tuberculosis.
REPORT OF COMMITTEE ON TUBERCULOSIS


Your Committee on Tuberculosis has given careful consideration to the status of the national bovine tuberculosis eradication project. It is recognized that the percentage of cattle reacting to the tuberculin test in fiscal year 1957 is higher than for any similar period since 1950. While the slight increase in infection rate may be explained by the fact that we are tracing more diseased animals and testing more suspicious herds, it does suggest that we are hardly holding our own in attaining our goal of complete eradication. We have given special consideration to the following problems that face us as we strive to eliminate this disease. We know there are others just as important, or more so, than those listed here. Many of them have been repeatedly brought to the attention of this Association at prior meetings.

1. Diligent studies are presently being made in some states in an attempt to develop satisfactory procedures for identifying cattle to facilitate tracing those found diseased to the farm or ranch of origin. These studies and trial procedures that may be developed must in most cases be coordinated between states. In the interest of promoting more concerted effort in this direction, it is recommended that this Association select a member from each of the eight United States areas as presently designated by the Animal Disease Eradication Division, United States Department of Agriculture, to work with the respective State and Federal cooperating officials, the A.D.E. Area Director and other appropriate agencies to promote improved methods of identifying cattle, so that those found diseased may be satisfactorily traced. It is further recommended that this special committee of eight take into consideration the specific conditions that exist in the different areas as they relate to the over-all national identification picture and make a report of accomplishments to this Association next year.

2. Proposals for changes in the uniform methods and rules for tuberculosis eradication especially as they relate to reaccreditation of areas based on standards of efficiency in tracing cattle found tuberculous at slaughtering establishments have been considered. Your Committee is cognizant of the need for additional consideration of these proposals, but recommends that such changes be deferred pending the development and instigation of methods that will make it possible to trace a much higher percentage of such animals than are now being traced. We do not concur with the proposal for less testing at the present time in areas where the incidence of tuberculosis is relatively high.
3. The effective contributions to the program that Public Health officials have made in sponsoring laws and regulations which limit the sale of dairy products to herds free from tuberculosis is recognized. In the interest of uniformity of regulations and procedures, it is recommended that the officials of the United States Public Health Service and the United States Department of Agriculture work together for the purpose of drawing up mutually satisfactory proposals for amendments to the Uniform Methods and Rules and to the Milk Ordinance and Code recommended by the Public Health Service as it refers to bovine tuberculosis. It is further recommended that the proposals agreed upon by these two agencies be jointly presented to this Association for consideration next year.

4. The “no-gross-lesion reactor” is considered a problem in many areas and is especially embarrassing and costly in others. Your Committee believes that this problem should have more careful consideration, and recommends that the Agricultural Research Service, United States Department of Agriculture, instigate further studies with a view to carrying on additional investigations and research in an effort to find means for reducing the severity of this problem.

5. Frequent references have been made to the lack of training and experience in tuberculosis eradication work by veterinarians accredited for official work. This is generally recognized and reflects to some degree on the present policies and procedures of State and Federal Livestock Sanitary officials. It is recommended that before a veterinarian is recommended for accreditation, that the cooperating officials assure themselves that he is sufficiently well trained and indoctrinated to properly perform their official activities in connection with tuberculosis eradication.
SYSTEMIC INSECTICIDES FOR CONTROL OF CATTLE GRUBS AND OTHER LIVESTOCK INSECTS

A. W. LINDQUIST, B.S., M.S., Ds.C.*

Nearly everyone who has conducted investigations on the control of cattle grubs has been aware of the need for improved ways of dealing with this costly livestock pest. Growers, packers, processors, and others have all voiced a need for a means of control better than the currently recommended rotenone sprays. Not only do rotenone sprays seldom provide highly effective control, but they have to be applied two or three times during the coldest and most severe season of the year. Our research objective has therefore been to find a better material, preferably a systemic insecticide that will destroy the young grubs within the body of the host before the flesh is injured and before the hide is perforated by the larvae. A material that would perform in this manner and be safe for general use would mark a great advancement in grub control.

Research by State, Federal, and industry workers has now reached a point where the control of cattle grubs by the use of systemic insecticides appears assured. The purpose of this paper is to explain how investigations on systemic insecticides are conducted, review research done during the last few years, and discuss their current status for the control of several livestock pests, but particularly the cattle grub.

METHODS OF CONDUCTING RESEARCH

The idea of internal use of insecticides for control of insect pests of animals has been publicized recently, but investigations along this line go back many years. In the early 1920's many proprietary products were purported to control insects when added to the feed of animals, particularly poultry. Parman et al. (1928) experimented with dozens of chemicals against natural infestations of chicken lice and fowl ticks, but concluded that none of them would control external parasites. Other investigators also reported negative results, and it was not until the middle 1940's that any ray of hope could be seen for the use of systemic insecticides. Work at the Orlando, Fla., laboratory of the United States Department of Agriculture (Lindquist et al. 1944, Knipling et al. 1948) indicated for the first time that certain internally administered chemicals would kill bloodsucking insects feeding on laboratory animals.

Efforts in this type of experimentation were gradually expanded. There existed a need for simple, economical laboratory methods of rapidly testing numerous compounds for systemic activity. This was especially true for

tests with cattle grubs. The grubs have a life cycle of nearly one year, and they cannot be reared in an artificial medium or on laboratory animals. The use of large numbers of cattle for screening numerous compounds is obviously too expensive. Several years ago at the Kerrville, Texas, laboratory a technique was developed in which first-instar grubs obtained from gullets of cattle at packing plants were implanted in mice, which were then treated with chemicals, either orally or by subcutaneous injection, and sacrificed after several days to determine mortality of the grubs. Hundreds of compounds were tested, but none of them showed any promise. It is possible that the test method was faulty, in that the young grubs placed in a rodent host responded differently than in the natural bovine host.

About 1950 a low-cost, simple, and fairly reliable screening procedure was developed at Kerrville, which uses guinea pigs as host animals. Guinea pigs are weighed, and the hair on the abdomens is removed. Small plastic containers are attached to the shaved areas and 10 starved nymphal lone star ticks (Amblyomma americanum (L.)) are placed in each one. Twenty-four hours later the guinea pigs are wounded and infested with about 50 newly hatched larvae of the screw-worm (Callitroga hominivorax (Cqr.)). When these larvae are 24 hours old, the pigs are treated with candidate insecticides. A dose of 100 mg./kg. is administered to two pigs, orally to one and subcutaneously to the other. At intervals thereafter starved stable flies (Stomoxys calcitrans (L.)) are allowed to feed on the pigs and then held for 24 hours to determine if they are affected by the blood they ingest. If the screw-worms are killed by the insecticide, the pigs are reinfested and similarly tested every 24 hours to determine lasting effects of the treatment. The ticks are held after engorgement to determine whether they die or live long enough to molt.

If this technique shows that a chemical has systemic properties, it is then tried on sheep, goats, and cattle. Those compounds that destroy artificial screw-worm infestations in these animals are viewed as good candidates for testing against cattle grubs. If the material has not shown undue toxicity to guinea pigs, cooperating veterinarians determine doses that may be safe to use on livestock and study the toxic symptoms resulting from the various methods of administration.

Following these studies groups of eight to twelve cattle having a good history of exposure to heel flies, the adults of cattle grubs, and likely to be infested with grubs are selected for further evaluation of the compound. Treatments are usually made well in advance of the appearance of grubs in the backs, and several months elapse before the results are apparent. If very few or no grubs appear in the treated animals but good numbers are found in the untreated checks, and if no serious toxicological symptoms are indicated, there is reason for optimism and verification tests with more animals are undertaken the next season. At least two and usually three or more years of wide-scale trials under different climates and other conditions are therefore required to determine the material's entomological effectiveness. In the meantime studies are conducted to determine if it leaves an undesirable residue or causes off-flavor in the meat and other edible tissues.
TESTS WITH CHLORINATED HYDROCARBON INSECTICIDES

Toledo and Saur (1950) reported from South America that BHC administered subcutaneously in cattle would destroy larvae of the human bot fly (Dermatobia hominis (L., Jr.)). This suggested the need for evaluating various chlorinated hydrocarbon insecticides as internal treatments for control of cattle grubs and other insects.

Our studies (Lindquist et al. 1953) showed that dieldrin, aldrin, lindane, and heptachlor injected subcutaneously into mice killed deer flies (Chrysops discalis Will.) and mosquitoes (Aedes dorsalis Meigen) taking blood meals, but when injected into cattle these insecticides gave poor results against natural populations of these insects. McGregor and Radeleff (1954) and McGregor et al. (1955) obtained good control of grubs in the backs of cattle with dieldrin, aldrin, and lindane. Roth and Johnson (1955) obtained high kills of cattle grubs with two subcutaneous injections of dieldrin at the rate of 25 mg./kg. However, none of these materials destroyed young grubs in the bodies of the cattle. Furthermore, they were considered unsatisfactory because large amounts of residues accumulated in the fat.

TESTS WITH ORGANIC PHOSPHORUS INSECTICIDES

A few organic phosphorus insecticides, such as Diazinon, Dipterex, and Chlorthion, were found which performed in a way similar to the chlorinated hydrocarbons when administered internally; i.e., they destroyed grubs after they had migrated to the back and cut holes in the hide. They did not kill small larvae in the host, but did destroy the mature larvae encysted in the skin. Young dipterous larvae of most species are much easier to kill than more mature stages when the insecticides are used directly on the insects. Very likely the mode of action and distribution account for lack of kill of young larvae within the animal. However, since they failed to kill the young larvae which would necessitate two or more treatments the materials were not regarded as particularly promising.

Dow ET-57.—A major break-through in systemic research on cattle grubs occurred in 1954, when the systemic action of Dow ET-57 was discovered by workers at the Corvallis, Oreg., and Kerrville, Tex., laboratories of the United States Department of Agriculture. A great deal of laboratory and large-scale experimental work has been done with this compound at both these laboratories, in various states and Canada, and by the producer. When it was given orally several weeks or months before grubs appeared in the back, the young larvae in the host apparently were destroyed. This was the first insecticide found to kill young grubs in the host before they appeared as lumps on the back. It was found effective against both species of grubs, Hypoderma bovis (L.) and H. lineatum (De Vill.). By 1955 we were getting closer to a practical method of combating cattle grubs. Oral administration at rates from 90 to 110 mg./kg. (about 1 ounce/600 pounds) of body weight after the heel fly season has given from 85 to 100 percent control.
Preliminary research by State and industry workers has also indicated that the insecticide may be effective when added to the feed at low levels of about 5 p.p.m. for varying periods. However, these promising investigations have not progressed sufficiently to determine the safety and effectiveness of low-level feeding under a wide range of conditions.

Toxicology research conducted at Kerrville by R. D. Radeleff of U.S.D.A. Animal Disease and Parasite Research Division and others indicates that Dow ET-57 will not harm cattle when used at the recommended dosages, although occasional symptoms of fleeting duration occur, such as bloating, increased salivation, leg weakness, and diarrhea. Doses several times that required for grub control have not caused any mortality of animals even though the higher doses are detrimental to them.

In order to obtain information on the uptake of the insecticide by the grubs, residues in tissues, and absorption, degradation, and elimination by the bovine host, the compound was labeled with P³² by C. C. Roan and associates at Kansas State College, and used in studies by W. E. Robbins, T. L. Hopkins, G. W. Eddy at Corvallis, and J. N. Kaplanis and R. C. Bushland at Kerrville. The tagged material was administered orally at the rate of 100 mg./kg.

The Corvallis group found that three hours after treatment the material appeared in the blood at a rate of 1 /ug.-equivalent per milliliter (1 p.p.m.) and that the radioactivity increased rapidly to a maximum of 22.8 /ug.-equivalent at 12 hours. Paper chromatographic studies of blood extracts demonstrated about 98 percent of the insecticide present to be unchanged ET-57 and two percent probably the oxygen analog. During an 11-day period about 86 percent of the administered dose was accounted for in the urine, the peak amount occurring between six and 30 hours. Chromatographic analysis showed only trace amounts of the parent compound, indicating rapid and extensive breakdown. After 14 days the omental fat contained 6.9 p.p.m. of unchanged ET-57, as determined by partition chromatography and bioassay.

Simultaneous work at Kerrville showed similar results except that the omental-fat samples were taken three days after treatment and they contained 50 p.p.m. of unchanged ET-57. It is likely that in 30 days most of the insecticide would be eliminated from treated animals, and further studies by the producer indicate that a 60-day waiting period between treatment and slaughter should assure absence of residues. In order to determine the fate of the grubs, treated animals were slaughtered, but no cattle grubs were found in the back, the gullet, or other tissues.

Much research will be necessary to determine how the insecticide or its degradation products destroy the grubs. It is possible that direct kill is not effected, but rather a disturbance to the insect's enzymes regulating migration or other activities. For example, the inhibition of the collagen-collagenase system, which has been found by Lienert and Thorsell (1955) to play a part in the forward movement of grubs in cattle tissue, could well result from the use of systemic insecticides.
At Kerrville screw-worms were allowed to feed on animals treated with the tagged insecticide. The radioactivity was first found in the maggots after three hours and amounted to 0.26 /ug.-equivalent per gram. There was a progressive increase to 5.21 /ug.-equivalent per gram, or less than 0.1 /ug-e-quivalent per larva, at the ninth hour.

Stable flies allowed to suck the blood of treated animals were affected. From 82 to 100 percent of them were killed during the first 36 hours, with a peak mortality at eight hours. There was a close correlation between the fly mortality and the radioactivity in the blood.

Bayer 21/199.—Another interesting compound found to possess systemic insecticidal properties by the U.S.D.A. researchers is Bayer 21/199. This organic-phosphorus insecticide has the unique property of acting systemically in controlling certain insects when applied as an external spray. It is effective against cattle grubs when animals are thoroughly sprayed once with a 0.5 to 0.75 percent concentration several weeks or months before the grubs encyst in the back. It does not destroy grubs when given orally to cattle. It has 24- to 48-hour systemic action when applied as a spray on bloodsucking stable flies and on screw-worms. Insects are also killed when resting on animals sprayed with the material. This insecticide therefore acts by direct contact as well as through systemic action.

When applied as a spray to livestock, Bayer 21/199 gives protection as a contact insecticide against screw-worms up to 14 days. It destroys screw-worms in wounds and also new larvae that develop in new or old wounds several days after treatment. There is evidence that it acts systemically for a short period, but its effectiveness apparently is due mostly to the insecticide flaking off the hair and falling into wounds. The material is highly potent, since 0.25 to 0.5 percent concentration kills screw-worm larvae when they are dipped momentarily. Should this insecticide prove to protect all classes of livestock against screw-worm attack for two weeks under various weather conditions, it will provide a great advance in control of this serious insect pest.

Preliminary work with P32-labeled Bayer 21/199 has shown some especially interesting results. Cattle in metabolism cages were treated with two-percent sprays in amounts that gave approximately 40 to 60 mg./kg. The maximum radioactivity in the blood of one animal occurred on the sixth day and amounted to only 0.28 /ug.-equivalent per milliliter, which is in marked contrast to the 22.8 obtained with ET-57. Studies with extracts of the radioactive blood showed that the parent compound had been metabolized. Only low levels of radioactivity were detectable in the urine. At the end of two weeks only 6.27 percent of the dose was accounted for in the urine of one animal treated with an emulsion at a rate of 52 mg./kg. Of significance was the fact that the hair of the animal retained large amounts of radioactivity throughout a six-week study period.

This work indicates that Bayer 21/199 is poorly absorbed through the skin and slowly eliminated. Most of the small amount absorbed is metabolized to other compounds, but it is not known if these degradation products kill
the grubs in the tissues. Since the orally administered insecticide is apparently ineffective against grubs, it is concluded that the digestive tract destroys the insecticidal qualities of the material or otherwise prevents action of the insecticide on the insect.

American Cyanamid 12880.—This compound, known chemically as O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate has shown promise in preliminary tests. Oral doses of 10 mg./kg. have given good kills of grubs in the backs of cattle, and there is some evidence of its effectiveness when administered intramuscularly. The effectiveness of the material when given before the grubs are visible has not been thoroughly evaluated, but apparently good kill is obtained. This insecticide has systemic effects at considerably lower doses than the other organic-phosphorus compounds. However, it does produce toxic symptoms at lower doses, and more research is needed to determine its advantages and limitations.

Other systemic materials, primarily organic-phosphorus compounds, are in various stages of study, and the prospects are bright that safer and more effective compounds will be found. Industrial chemists are synthesizing hundreds of new materials, many of which are related to those now showing systemic properties. Our Department and State workers are cooperating with industry in this research. It is believed that some of them will prove even more useful than those now available.

SYSTEMICS AGAINST INSECTS OTHER THAN GRUBS

Up to this point I have made little mention of the effect of systemics against biting flies, mosquitoes, lice, and ticks. The chlorinated hydrocarbon insecticides mentioned earlier were effective for several days against mosquitoes and tabanids but are not practical to use on livestock. The organic-phosphorus compounds killed bloodsucking flies for a day or two, but this is not long enough for ordinary use. The problem of finding a safe insecticide that will continue to kill bloodsucking flies for long periods is indeed formidable, especially when excessive residues in meat and milk must be prevented. The use of systemics for such pests most likely will involve continuous low-level intake through feed or mineral supplements with a required waiting period between last intake and slaughter.

Neither Dow ET-57 nor Bayer 21/199 has been especially effective as a systemic against cattle lice. Because a systemic is not likely to destroy louse eggs, a long-lasting material or continued intake in feed for several weeks or repeated treatments 14 to 18 days apart would be necessary to destroy the nymphs hatching from eggs.

On a worldwide basis, there is a real need for effective systemics against the various ticks, especially those transmitting livestock diseases. The same problem occurs as with louse control—i.e., the finding of compounds having long-lasting residual effects or safe materials that can be frequently administered. As yet no systemic has shown much effect on ticks in experiments at Kerrville.
PRESENT STATUS OF SYSTEMICS FOR GRUB CONTROL

In late September the United States Department of Agriculture and the Food and Drug Administration approved provisional registration of Dow ET-57 for limited use to control grubs. The compound will be available this fall only in local areas in four States—Nebraska, Iowa, South Dakota, and Wyoming. Many people who have been anticipating nationwide availability will be disappointed at the local nature of this distribution. However, its purpose is to permit a proper evaluation of problems arising from rather wide-scale grower usage of an entirely new material and method of application in cattle grub control and to determine the degree of grub control that can be achieved on a practical scale. In such a situation it is considered wise and desirable to proceed slowly and with some caution so as not to jeopardize the over-all objective of systemic insecticides research and use. State, Federal, and industry workers plan to watch the situation and look for problems that might arise, as well as for general effectiveness of this insecticide in trial areas.

As with any insecticide, certain precautions are necessary. For best results treatments should be made after the heel fly season and before grubs appear in the back. Usually this is June through October in southern areas and September through December in northern areas, but local conditions will affect the time of treatment and State and Federal entomologists should be consulted. Best results have been obtained by early treatment; late treatment when grubs are already in the back is not recommended.

Only one treatment is required and the rate of application is approximately one ounce for each 600 pounds of body weight. The material may be given in the form of a bolus with a balling gun or dispersed in water and administered with a syringe or bottle as a drench. Cattle should have free access to regular feed and water before and after treatment.

To avoid contamination of meat, cattle should not be treated within 60 days of slaughter. Lactating animals should not be treated at any time.

For this fall the Entomology Research Division suggests the use of this compound by growers in states where it is commercially available and in accordance with the recommendations given. In the meantime cooperative research is being continued in efforts to make available systemics for general use.

LITERATURE CITED


REPORT OF THE COMMITTEE ON PARASITIC DISEASES


It is impossible to estimate the losses to the livestock industry by the ravages of external parasites. Reports from various agencies on the estimated damages have shown far greater losses from these external parasites than from any other types. Most of the damage is not visible and therefore only an estimate of these losses can be made.

The warble or heel flies, *Hypoderma bovis* and *Hypoderma lineata*, are two of the species that cause damage both to the livestock raisers and to the meat and tanning industry. The A.R.S. in a special report January, 1957, states that “Cattle grubs and heel flies are responsible for sizeable losses to the American livestock industry. Actual dollar losses have been estimated by industry groups to range between $100 million and $300 million annually.” These two flies, in order to complete their life cycles, annoy and damage the body of cattle severely. All during the time the flies are laying their eggs and the larvae are wandering in the body, the livestock raiser is “footing the bill” for the completion of the life cycle; while the meat packer and tanner “pay” later for their share of the cost of the life cycle in “grubby carcasses” and ruined hides.

Every stockraiser is familiar with the annoying attacks of the flies as they lay their eggs on cattle causing stampeding and other violent reaction by the animals to avoid the flies. This excitement, worry, and over-exertion results in loss of weight and milk. As the larvae penetrate the skin, the irritation causes increased annoyance, resulting in poor grazing, loss of weight and milk production. When the larvae migrate through the muscle and tissue the pain must be excruciating, especially when the larvae cut holes through the skin in the back.

Most of the above losses can only be estimated, but the losses to the meat packer and tanner are clearly visible. The meat packer encounters specific losses by damages to muscle tissue and organs by the larvae. In the experience of those that have done Meat Inspection or worked on trimming carcasses, the losses are clearly outlined. During the late fall and winter many grubs are found in various organs, especially in the oesophagus and the muscles of the back. The most valuable part of the carcass, the subcutaneous tissue and muscular portion of the back, are infiltrated with encysted grubs causing greenish-yellow, gelatinous, edematous areas, and pus pockets that must be trimmed. In some animals the infestation is so heavy that much of the most expensive portions must be carefully trimmed lowering the carcass grade. In some instances the grubs are hidden deep in the
muscles and are overlooked by the trimmer or butcher. This has a psychological effect on the housewife when she finds the grub in a steak or roast. The partially eaten cooked roasts or steaks are brought back with angry indignation that such meat is sold; hurting future sales of meat and public relationship.

Severe economic losses are caused by the numerous holes and scar tissue left by the grubs in the most valuable part of the hide. In the A.R.S. special report, mention is made that “Hides and meat of infested animals are damaged during the latter part of the larval stages when they encyst in the backs of cattle. A third of the hides of the 18 million cattle slaughtered in the United States in 1948 contained five or more holes and were sold at a discount of a cent a pound. In the same year about 14 million pounds of choice meat were trimmed from carcasses to remove damaged areas.”

Many other reports have been given on the losses from various areas. For example, in 1952, Dr. I. H. Roberts of the Animal Disease and Parasite Research Division, United States Department of Agriculture, found that in one lot of 31 calves slaughtered in Oklahoma City, 24 were grub-infested. The total loss of $58.43, over $2.00 per head, was apportioned as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downgrading due to trimming</td>
<td>$22.59</td>
</tr>
<tr>
<td>Weight value of meat trimmed</td>
<td>17.60</td>
</tr>
<tr>
<td>Labor cost of trimming</td>
<td>.50</td>
</tr>
<tr>
<td>Dollar value of hide damage</td>
<td>17.74</td>
</tr>
</tbody>
</table>

These figures were furnished by the abattoir where the infested animals were slaughtered and processed.

In the past in order to help control these two pests many drugs, chemicals, and mechanical means have been used with a varying degree of success. Many thousands of dollars have been spent on “Quack” remedies such as mineral supplements and feed supplements that were supposed to kill cattle grubs when they arrive in the loin area. Hundreds of insecticides and sprays have been used on cattle to prevent the flies laying their eggs, but with very little success in either killing the fly or keeping them away from cattle.

Most of the successful methods were used on the basis that the vulnerable time in the warble flies’ life cycle was during the time the larvae were in the back and after they had cut holes in the hide. At this stage rotenone powders have been used with great success to destroy the “grubs” by contact. But the damage had already been done both to the livestock raiser, to the meat packer, and to the tanner. Destruction of the larvae did, however, cut down the numbers of flies that would appear the next year.

During the past 10 years much attention has been given to oral or subcutaneous administration of insecticides that are absorbed from the digestive tract and circulate through all the body tissues to destroy the larval stages in the body. Some of the insecticides used have been partially successful when fed or injected, but usually they did not kill the larvae in the tissue, and so were no better than rotenone. External application of insecticides has never been practical. Research workers for many years have been interested in
systemic insecticides usually given in feed supplements or rations to control external parasites of all kinds.

Hundreds of the newer chemicals have been tried experimentally by many investigators, Federal, State and commercial, to combat the cattle grubs and other external parasites systemically. Aldrin, dieldrin, lindane, chlordane and others have had very little effect against the grubs when administered orally or subcutaneously. Diazinon and Dipterex gave partial results but the dosage rates were close to the host toxic levels and not too successful.

Phenothiazine, after the success in low-level feeding for strongyle control, showed some promise of success in systemically destroying the grubs in the tissue, but subsequent experiments have failed to prove that phenothiazine is useful as an adequate systemic control of cattle grubs. A great deal of research work is still being done to evaluate the use of phenothiazine.

Several systemic insecticides have shown very promising results experimentally against the cattle grubs. One, a phosphate compound known as Dow ET-57 has been proven experimentally to kill the larvae while they are migrating through the body. Given orally at the rate of 100 milligrams per kilogram (1.6 ounces to a 1,000 pound animal) in a single dose, it will kill the larvae in the tissue before any damage can be done. This chemical administered orally, is absorbed from the digestive tract, circulates through the body destroying the larval stages in the muscles and tissues before they reach the back. In none of the numerous tests made was there any toxicity from the dosage given, and all taste-tests showed no change in meat flavor. The compound can be given orally either in a capsule, bolus or drench, but some experimental work is being done with a low-level feeding of the drug in mineral mixtures or feed supplements so that the intake is over a long period of time. The use of the material as a spray was not successful systemically except to kill the grubs in the back and was not as effective in preventing damage as when given orally.

Dow ET-57, to be called "Trolene," has been approved by the Food and Drug Administration in bolus form for treatment of cattle to control cattle grubs. Approval now has been granted for the sale of "Trolene" boluses both in Canada and the United States.

Another phosphorous compound, Bayer 21/199 shows great promise experimentally in controlling the grub. This compound shows promise as a systemic to destroy cattle grubs but unlike ET-57, it is absorbed when used as a spray. This type of compound used as a spray could be a more practical approach than by the time and labor consuming efforts to administer drugs orally. The chemical is absorbed through the animal's skin and then acts as a systemic to destroy the larvae before they reach the back.

CL 12880 (American Cyanamid) an organic phosphorus compound, has also shown gratifying initial results experimentally in the control of grubs.

Other compounds are still being tested for use as systemics against the larvae in the tissues with many companies testing various chemicals they feel are quite active against cattle grubs.
Experimental trials with Dow ET-57 resulted in the destruction of 100 percent of the first instar larvae of the nose bot fly, *Oestrus ovis*, in the head sinuses of sheep, but a lesser number of second and third instar larvae were destroyed. The dosage was 100 mg. per kg. Higher dosages may give more promising results in future experiments on the second and third instar larvae of the nose bot fly.

The Committee would like to point out that there is still much research work to be done on the eradication of various other ectoparasites by systemic insecticides and other means. Lice, ticks, mites, fleas and numerous flies, especially the *Tabanids* (horse flies), still present a great problem that must be solved.

The Committee also feels that much more intensive research work must be done, than in the past, on other parasites of domestic animals. They feel that intensive studies should be done on exotic parasitic infections so as to alert ourselves to possible dangers if any of these exotic species should ever gain a foothold on the American continent.

Still another problem is the constant inter-state shipment of parasitized animals, with modern transportation disseminating parasites widely. Unless preventive methods are taken early, newly-introduced parasites in free areas will cause enormous damage before they can be brought under control.
The poultry industry has grown tremendously in recent years. Farm income from poultry and poultry products is challenging other branches of the livestock industry for pre-eminence. Disease is the principal limitation on poultry production. The disease factor has become more and more important with the growth of the industry. The maintenance of large flocks in more restricted quarters, the establishment of the hatchery industry with its potential hazard of widespread dissemination of egg transmissible diseases, and the increase in transportation, have all tended to increase the losses from disease.

Unfortunately, the treatment, control and prevention of poultry diseases, unlike diseases of other domestic animals, has remained largely in the hands of persons with little or no veterinary training. The peddling of worthless and misapplied drugs, and the so-called "servicing" of diseased poultry flocks by untrained and improperly qualified individuals, has exacted a terrific toll from the poultry industry for generations.

Early in the development of the hatchery industry, it was found essential to control pullorum disease for satisfactory operation. This resulted in the adoption of the pullorum stages of the National Poultry Improvement Plan. The outstanding results of this program, has demonstrated the possibility of vast improvement in the control and eradication of disease when an orderly, systematic plan is available.

There is now a demand for the establishment of programs similar to the pullorum disease eradication plan now in effect, applying to other diseases. However, in many states, the pullorum disease control program is administered by lay personnel, who do not have the required medical training or the basic knowledge to formulate and carry out programs for the eradication of other diseases. Consequently, this demand by the poultry industry has not been met.

There is no basic difference between the control and eradication of diseases of poultry and similar procedures applying to diseases of other domestic animals. Each disease presents a separate problem and is complicated by the economic factors and established practices of the industry, which must be considered. It seems that the time has arrived when livestock disease control agencies must realize their obligation to the poultry industry and proceed in the same manner as with other species of livestock to protect the health of the poultry in their respective states.
Some of the immediate problems faced by a state livestock disease control agency in extending its service to the poultry industry are as follows:

(1) Legal authority. As stated above, in some states the operation of the pullorum disease control program is delegated to a separate agency from the livestock sanitary authority. In most such cases, persons without veterinary training operate the program. It seems imperative that this condition be amended to place the operation of all poultry disease activities under the authority of the State Livestock Sanitary Agency. In Minnesota, the State Live Stock Sanitary Board has included in their activities, the control of poultry diseases for some 50 years. Authority was based on an opinion of the State Attorney-General that the term "domestic animals" as used in the livestock sanitary code, included poultry. Also when additional legislation has been enacted from time to time, applying to the control of diseases of poultry, the legislature has provided for enforcement by the State Live Stock Sanitary Board; and in 1957, the Legislature went a step farther by enacting legislation defining the terms "livestock" and "domestic animals" as used in the livestock sanitary code, to include poultry.

(2) Confidence in the ability and interest of the State livestock disease control agency on the part of the poultry industry, is imperative if success in the control of disease is to be obtained. Because of the unfortunate disregard of poultry disease problems by many individual veterinarians and the resulting assumption of these activities by other agencies, this is a real problem in many states. A program of education and full cooperation with leaders of the poultry industry must be established. We have found the poultry industry exceedingly responsive to such activities. Industry leaders are fully aware of the disease situation and welcome assistance by a properly qualified state agency, if real interest and a spirit of cooperation is in evidence.

(3) Another difficult problem is the choice of personnel by the state agency to administer poultry disease problems. The poultry industry, like all other branches of livestock production, has certain economic problems, customs, and even language peculiar to that industry. Personnel delegated to poultry disease control, in addition to professional qualifications, must be able to talk the poultryman's language, and be sympathetic with his economic problems. Veterinarians with these qualifications, are not too plentiful, but can be found, and younger veterinarians can develop the necessary qualifications.

(4) As is true in most areas, one of the most difficult problems we have to face, is the procurement of money. During recent years, "economy" has been the watchword of many State Legislatures, and of course, properly so. Any activities are usually provided for only when justification in minute detail can be presented to the legislative appropriation committees. It has been found in this State, that the problems of finances must be discussed freely with industry leaders and their support obtained during the legislative session, in order to obtain the necessary public funds. We have found the poultry industry more than willing to furnish such support, and even to carry such part of the financial load as may be necessary, but it is evident that the operation of disease control programs, and the salaries of supervisory
personnel must be paid from some source, which is not in any way controlled by the industry which is supervised.

(5) One real problem that must be faced after confidence in the state agency is established with the industry, is to keep up with the demands for further extension of disease control activities. As one program is developed and put into successful operation, requests by the industry for tighter controls in programs under way and formulation of additional programs for other diseases are immediately forthcoming. State livestock disease control agencies must meet this challenge if we are to retain our place of leadership in protecting the health of our vast livestock industry, including that part engaged in the production of poultry and its products. We cannot afford to disregard these demands and must exercise all the energy and resources at our command to meet them.

There are, of course, the problems of enforcement common to all phases of livestock disease control, which are similar, if not identical to the same problems faced in the control of diseases of other livestock. These cannot be enumerated here and I am sure they are unnecessary to present to this Association at this time.

It is my firm conviction, that the control of poultry disease should be and must be made a major activity of all State livestock disease control agencies if we are to render the service which is expected of us. In most states there is no other state agency with the personnel and experience necessary to successfully administer disease control programs with all their ramifications, and only by duplication of activities, could any other such agency be properly qualified. The field for expansion of these activities widen, and the opportunity great. Every effort should be exerted to meet the challenge.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

M. S. Hofstad, Chairman, Ames, Iowa; A. Christie, Kingston, New Hampshire; J. P. Delaplaine, College Station, Texas; H. M. DeVolt, College Park, Maryland; E. M. Dickenson, Corvallis, Oregon; J. F. Witter, Orono, Maine; H. E. Goldstein, Columbus, Ohio; P. P. Levine, Ithaca, New York; J. W. Munn, Atlanta, Georgia; N. O. Olson, Morgantown, West Virginia; B. S. Pomeroy, St. Paul, Minnesota.

The chairman of this Committee wishes to express appreciation to other members of the Committee who contributed to this report.

No major problems have developed for the poultry industry this past year and work has progressed in solving some of our disease conditions. One of the outstanding contributions in the field of poultry diseases this past year was the finding that the virus of visceral lymphomatosis could be cultivated in tissue cell cultures. This should be a great aid in working with this disease complex. Chronic respiratory disease continues to be a major poultry disease problem, but our knowledge of this disease has progressed to the point where we should begin to think about field control work. The control of this disease is a formidable problem, but efforts should be increased in this area. Ornithosis in turkeys continues to be a public health problem and its importance has been emphasized during the past year by the Federal regulation restricting the interstate movement of flocks affected with ornithosis. The passage of the Federal “Poultry Products Inspection Act” in August of this year is a milestone in our continuing efforts to provide wholesome meat and meat products to our population. Compulsory poultry inspection should be favorable for increasing consumption of poultry meat. Effective January 1, 1959, the law applies to plants involved in interstate shipment of dressed fowl. Individual states may still have problems to solve with local plants not in “designated major consuming areas.”

RESPIRATORY DISEASES

Newcastle Disease. There have been no widespread outbreaks of Newcastle disease during the past year. Either the vaccines are doing an effective job or the flocks are not being challenged by field strains of the virus. There have been a number of reports on the effectiveness of Newcastle disease vaccines. The observations by Winterfield et al. (1) indicate that the B1 vaccine strain, given in drinking water to chicks under two weeks of age with passive antibodies, induces a poor immune response. Even revaccination at four to five weeks after initial vaccination at four days of age resulted in an erratic immune response. When vaccination was done at three weeks of age, however, a good immune response was obtained. Bankowski et al. (2) also demon-
strated interference of passive antibodies with immunity to B1 vaccine. Experiments have also demonstrated the importance of an adequate dosage of drinking water vaccine for maximum immune response (3). Studies (4), comparing the B1, F and LaSota vaccine strains, found the LaSota strain to induce a slightly better immunity in chickens than the other two strains, but it also was more pathogenic and had greater spreading ability than the B1 and F strains.

A study by Dardiri et al. (5) revealed formalin-killed Newcastle disease vaccine to protect 98 percent of broilers to a challenge inoculation. Similar groups given water vaccine at five days of age caused only 28 percent to resist challenge at 12 weeks, but a group revaccinated at four weeks of age obtained a booster effect so that 94 percent were immune at 15 weeks. The dust vaccine induced an immunity which protected 70 percent and 100 percent of the broilers after one and two doses respectively. Another study (6) revealed the F strain to produce no bad effects and induced an immunity in three-week-old chicks which lasted 90 days. Clark et al. (7) found the aqueous humor of chickens to be a frequent source of Newcastle disease virus and this may be an important finding from an epizootiological standpoint.

**Infectious Bronchitis.** Avian infectious bronchitis appears to be effectively controlled. Widespread use of the modified, live virus vaccines have no doubt had a part in the decreased incidence of this disease in laying flocks. There is little reported work on the effectiveness of bronchitis vaccines, particularly on the duration of immunity.

Infectious bronchitis has recently been reported from Japan (8) and the Netherlands (9). Studies by Sevoian and Levine (10) found infectious bronchitis to permanently damage the reproductive tract of laying chickens in controlled experiments. Broadfoot et al. (11) found that the reproductive tract was apparently damaged from infectious bronchitis if antibody-free baby chicks acquired the disease during the first two weeks of life. This frequently resulted in so-called false layers. Hutt et al. (12), however, have found other causes for false layers in chickens. Recent studies (13, 14) have demonstrated at least three immunologically distinct strains of infectious bronchitis virus. No work has been done to determine how prevalent these other strains are in the field; however, it does emphasize the importance of using vaccine strains which will immunize against the most prevalent field strains. Levine (15) has studied the distribution of P32 and S35 in normal and bronchitis infected embryos and has found a marked difference. Domermuth and Edwards (16) have demonstrated the virus by electron microscopy in infected tissues. The virus was calculated to be 178 mu. in size which is considerably larger than that previously found for free virus.

**Chronic Respiratory Disease and Infectious Sinusitis.** On the etiology of chronic respiratory disease (CRD) it has been found that at least two immunological types of the organism exist (17, 18). This will add to the problems of control. The isolation of non-pathogenic pleuropneumonia—like organisms (PPLO) (19) from chickens and turkeys emphasizes the problems of diagnosis. The bacterial flora of the respiratory tract of chickens affected
with CRD has been studied (20, 21, 22) and found to consist of a variety of organisms, but chiefly Gram-negative bacteria the majority of which belong to the coliform group. Studies have continued on better methods of isolating the causative PPLO, *Mycoplasma gallinarum*. A modified Grumble's medium was the choice of one group (23, 24) and a minced embryo medium was found simple and suitable for isolating PPLO by Adler (25). Additional proof of egg transmission of infectious sinusitis was reported (26).

Treatments for CRD have been reported by several groups. A well controlled study by Olesiuk et al. (27) found none of 10 products effective against uncomplicated PPLO infection. However four of these (Chlortetracycline, furazolidone, magnamycin and oxytetracycline) exerted a slight inhibitory effect on the course and severity of the disease. Hamdy et al. (28) found erythromycin to be an effective antibiotic against PPLO in vitro and in ovo and also found it to be effective in treating experimentally infected turkeys. Streptomycin continues to be a popular and widespread treatment for breeding flocks in an attempt to control egg transmission. There is need for controlled experimental work in this area to evaluate this method of treatment and control.

Crawley and Fahey (29) found the hemagglutination inhibition (HI) test useful in detecting infected flocks and suggested its use in field control of the disease. The antigen used is a stable, dried product. The use of the HI test was found to be a sensitive test for detecting infection in turkeys for as long as 18 months after the disease started (30). Dunlop and Strout (31), using the serum plate agglutination test for detecting PPLO infection, were successful in obtaining some flocks of PPLO-free chicks from infected breeder flocks by treating with dihydrostreptomycin to suppress egg transmission and raising the chicks in small, isolated groups.

*Laryngotracheitis*. While this disease is effectively controlled by proper vaccination, outbreaks of the disease still occur (32, 33, 34). Some of these outbreaks have been in supposedly vaccinated flocks (32, 34) and one outbreak (34) was studied and found to be due to failure of immunity to develop following use of a vaccine with \(<10^{-1}\) titer. This points out the need for vaccine standardization. Indirect transmission by man, rat, crow and dog was apparently responsible for one series of outbreaks (33). Cover and Benton (35) have continued to work with their avirulent strain of laryngotracheitis virus, and one additional isolation of an apparently mild strain was made by Satriano et al. (36). Hitchner and White (37) studied virus infectivity for embryos by four routes and found the dropped CAM technique to give titers of two logs greater than the other methods. They also found sinus inoculation a satisfactory method of challenge.

*Ornithosis*. A coordinated research program under the direction of Animal Disease and Parasite Research Division has been under way in California, Minnesota, Oregon and Texas. The work has been directed toward the development of new and improved diagnostic techniques as well as better understanding of the transmission, treatment and control of this disease. The development of a direct complement fixation test for chicken and turkey serums
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has been reported by Brumfield (38). A study group met at Texas A. & M., College Station, Texas, in June, 1957, to establish a standard test for ornithosis that may be used as a reference test. Davis et al. (39) were unable to recover the ornithosis virus from eggs laid by turkey hens in the viremic stage of the disease.

FOWL CHOLERA

Although fowl cholera has not appeared in epizootic form, its presence in most of the prominent poultry producing areas must be considered in diagnosis. The variable pathogenic nature of different isolates of Pasteurella multocida has received considerable attention in the past, and these differences need to be taken into consideration for most effective handling of the disease.

Acute fowl cholera infection may manifest itself in turkeys with gross pathological changes similar to those seen with erysipelas. In these cases, in addition to subcutaneous hemorrhages, a swollen, turgid tubular leader that at one time was considered pathognomonic of erysipelas may occur. This emphasizes the importance of cultural diagnosis.

In the field of immunization, Yaw and Kakavas (40) report on laboratory trials with a crude preparation of capsular polysaccharides from a Type 1 strain of \P. multocida\ and a somatic antigen of the B variant organism as providing a high degree of protection in chickens against challenge with the virulent culture.

FOWL POX

In general fowl pox is satisfactorily controlled by immunization with fowl pox vaccine by the “stick” method. Recently, however, Edgar and Bond (41) studying the duration of immunity found that some birds vaccinated at eight weeks of age with commercial vaccine were not fully immune to challenge 60 and 90 days later. A pigeon pox vaccine for “stick” application has recently appeared on the market. Goldhaft (42) has emphasized that the greater the size of the area vaccinated the better the immunity in the case of pigeon pox vaccination. Recently Seeger and Price (43) have demonstrated the unreliability of the immunity from pigeon pox vaccination.

AVIAN LEUKOSIS COMPLEX

Apparently the first visualizations of the virus particles of leukosis was reported by Burmester et al. (44). Electron microscope studies of sections of leukotic tissues disclosed large osmiophilic particles 90 microns in diameter. A cytopathogenic effect of leukosis RPL 12 virus on chick embryo liver tissue cultures was noted by Fontes et al. (45). Tissue culture propagated virus produced leukosis in inoculated birds. Sharpless, Defendi and Cox (46) reported similar results.

An analysis of data by Cole and Hutt (47) indicated that there was no relationship between age of parents and leucosis incidence in the progeny. On the other hand, Burmester and Waters (48) found that insofar as egg
transmission of leucosis virus was concerned, there was a reduction of rate of shedding virus as the hens got older. However, there was variation in the age when this reduction in virus shedding occurred.

That leukosis virus is eliminated in the saliva and feces of infected and carrier birds was demonstrated by Burmester (49). These methods of dissemination added to the shedding of virus in the egg play significant roles in transmission of the disease to susceptible stock.

Burmester et al. (50, 51) demonstrated that chicks from dams previously injected intraperitoneally with live virus were significantly more resistant to virus challenge than chicks produced by the same dams prior to immunization. Other methods of injecting the dams produced definite increases in resistance in the chicks. It was found (Burmester et al. (52)) that within limits, the severity of visceral leucosis in injected birds varied with the size of the dose.

Beard (53) published an exhaustive account of the physical, chemical and serological characteristics of the myeloblastosis virus.

**AVIAN ENCEPHALOMYELITIS**

**(EPIDEMIC TREMORS)**

The most recent significant contribution to our knowledge of the epizootiology of avian encephalomyelitis was made by Taylor et al. (54). They produced evidence indicating that laying hens while being trapped had experienced an unexplained drop in production. Progeny from eggs laid immediately prior and during the slump came down with epidemic tremors. Simultaneously, Schaaf and Lamoreux (55) announced that chicks hatched from pullets developed epidemic tremors while those from old hens did not. Further, they showed that a significant decrease in tremor incidence resulted when the pullets were vaccinated via the wing web with live, glycerinated avian encephalomyelitis virus.

Jungherr et al. (56) succeeded in adapting the virus to chick embryos by the intraocular inoculation route. Wills and Moulthrop (57) confirmed these results and succeeded in effecting virus growth by yolk sac inoculations of chick embryos.

The first naturally occurring outbreak of avian encephalomyelitis (or a similar virus disease) in birds other than chickens was described by Mathey (58). Mongolian pheasants were infected on a farm where epidemic tremors had occurred previously in chickens.

**SALMONELLOSIS**

**Pullorum Disease.** Progress has continued in reducing pullorum disease as a problem in turkey and chicken flocks. The voluntary programs of the National Poultry and Turkey Improvement Plans have been largely responsible for the progress in eliminating pullorum disease. In 1956-57 season it was estimated that 59.5 percent of the chicken hatcheries participated under the plan with very little change from the 1952-53 season. Considerable
progress has been made in the participating hatcheries qualifying for United States Pullorum-Typhoid Clean status; 86.7 percent of chicken hatcheries and 99.5 percent of turkey hatcheries have so qualified.

The results of the program are indicated in Table I (59).

Chickens and turkeys officially tested for pullorum disease, number and percent of reactors 1954-1957.

<table>
<thead>
<tr>
<th>Year*</th>
<th>Chickens Tested Number</th>
<th>Reactors Number</th>
<th>Reactors Per Cent</th>
<th>Turkeys Tested Number</th>
<th>Reactors Number</th>
<th>Reactors Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1954</td>
<td>37,766,375</td>
<td>88,918</td>
<td>0.24</td>
<td>2,946,586</td>
<td>2,641</td>
<td>0.09</td>
</tr>
<tr>
<td>1955</td>
<td>39,288,860</td>
<td>40,316</td>
<td>0.10</td>
<td>2,918,037</td>
<td>1,007</td>
<td>0.03</td>
</tr>
<tr>
<td>1956</td>
<td>36,112,781</td>
<td>25,529</td>
<td>0.07</td>
<td>3,310,470</td>
<td>1,014</td>
<td>0.03</td>
</tr>
<tr>
<td>1957</td>
<td>40,614,440</td>
<td>18,369</td>
<td>0.045</td>
<td>3,828,755</td>
<td>2,197</td>
<td>0.06</td>
</tr>
</tbody>
</table>

A report (60) giving a digest of Laws and Regulations affecting the sale of hatching eggs, chicks and poults indicated that 17 states had legal pullorum requirements which applied to all hatching eggs, chicks and poults shipped into the states. Six states required a permit on all imported shipments. Thirteen states require that all hatching eggs, chicks and poults shall originate from flocks or hatcheries that have a United States Pullorum-Typhoid Passed or Clean classification or from flocks that have met comparable pullorum requirements under supervision. All this indicated that positive action has been undertaken in some states to develop an eradication program for pullorum disease. At the annual meeting (1957) of the North Central Poultry Disease Conference a resolution was passed that a Federal regulation be developed on the interstate shipment of hatching eggs, chicks and poults to require participation under the disease phase of the plans or have met comparable pullorum requirements under supervision. The ultimate goal of complete eradication of pullorum disease from the United States should be considered. After July 1, 1957, the only official rapid, whole blood, plate antigen under the national plan is the polyvalent K antigen.

Sieburth (61) has reported on the use of indirect hemagglutination test in Salmonella infections of chickens and found the hemagglutinin titers appeared earlier, were consistently higher and persisted longer than agglutinin titers. The use of a polyvalent indirect hemagglutination test was investigated in experimental infections with encouraging results. The next National Plans Conference will be held at Louisville, Kentucky, June 24-27, 1958. Proposed changes must be submitted to Animal Disease Research Division, Beltsville, Maryland, by March 25, 1958.

Paratyphoid. At the recent meeting of the North Central Poultry Disease Conference considerable discussion pertained to the development of an official testing program for typhimurium. Several states in the north central region have been testing turkeys on a voluntary unofficial basis for several years with encouraging results.
Sieburth (62) reported that furazolidone at 0.005 and 0.01 percent suppressed the formation of agglutinins but not the formation of indirect hemagglutinins to *S. typhimurium* in orally inoculated chickens. The use of furazolidone in breeding flocks should be delayed until the completion of the pullorum and paratyphoid testing programs because of the hazard of suppressing the development of agglutinins by carriers.

**INFECTIOUS HEPATITIS**

Delaplane, Smith and Moore (63) described a new disease of chickens and characterized the tissue changes. An agent cultivable in chick embryos was isolated and found resistant to aureomycin, terramycin and penicillin. The agent was sensitive to streptomycin. Serum neutralization tests could not be done because of the low titer (measured by embryo lethal doses) of the agent. A similar disease was encountered in Massachusetts by Winterfield and Sevoian (64). They propagated the causative agent in chick embryos and found that it was sensitive to chlortetracycline, oxytetracycline, magnamycin and dihydrostreptomycin sulfate.

**ERYSIPelas IN TURKES**

Although the infection appears to be endemic in many areas of the United States where turkey production is of commercial significance, reports of outbreaks within these areas have been spotty. The disease in turkeys probably reached its greatest prominence in the late 1940's in the Pacific Northwest (Oregon, Washington) when many ranchers producing large numbers of turkeys suffered heavy economic loss from annually recurring infections of young stock at about four months of age or older.

Anti-swine-erysipelas serum has not given the satisfactory results in turkeys that is generally obtained in swine. On the other hand, the administration of penicillin in almost any of its forms has proved to be a most effective treatment.

Several workers have reported on various ways to prepare *E. rhusiopathiae* or its products as an immunizing agent. Dickinson *et al.* (65) have reported on the use of a bacterin of *E. rhusiopathiae* that has provided reasonably good protection for breeding turkeys provided they receive two inoculations 60 to 120 days apart. This procedure has been extensively used in the Pacific Northwest and has greatly reduced the losses previously experienced in this region.

**HEMMORHAGIC SYNDROME**

This condition has not appeared as a major problem the past year. Experimentally it has been possible to produce hemorrhages by feeding various toxic materials: beta-aminoproprionitril (BAPN) (Barnett *et al.* 66), toxic fungi (Forgacs *et al.* 67) and feed from the field where the hemorrhagic syndrome appeared (Henderson *et al.* 68). Adding vitamin K or ascorbic
acid to the diet reduced hemorrhages but some antibiotics (200 p.p.m.) increased the incidence of hemorrhages in eviscerated broilers (Sanford et al. 69). Sulfaquinoxaline added to the ration did not increase changes of the hematocrit in mechanical bled birds but did in the controls (Bletner et al. 70). These studies further indicate the complicated nature of the hemorrhagic syndrome. The factor or factors involved in field outbreaks of the condition have not been established.

INFEKTIOUS SYNOVITIS

This disease has now been diagnosed in the major broiler producing areas of the United States. The incidence of infectious synovitis (IS) in the various areas appears to be cyclic. Considerable trouble is encountered in an area and then the disease becomes less of a problem only to occur again. The cyclic nature has not appeared to be related to any particular season.

Cover and Benton (71) found the IS virus in nearly all body tissues and organs of the body. It was not found in intestinal contents or bile. Following intravenous or intramuscular inoculation viremia was present in 48 hours and persisted for 10 days.

Thayer et al. (72) presented evidence that IS may be spread through fertile eggs. The disease consistently appeared in chicks obtained from a single breeding flock. The agent was recovered from six-day-old chicks.

Further experimental work indicates that certain antibiotics will control IS. Seeger et al. (73) gave 100 gm. or 200 gm. per ton of dietary chlorotetracycline to 28-day-old chicks exposed intramuscularly. The 100 gm. level permitted a slight development of synovitis whereas the 200 gm. level did not. In another experiment the deleterious effect of synovitis inoculum was significantly reduced by 100 grams of chlorotetracycline per ton of feed in terms of weight and mortality. High protein aggravated the mortality caused by IS; energy level and vaccination had no effect (White-Stevens et al. 74). In a comparison of tetracycline antibiotics, daily injections of chlorotetracycline, oxytetracycline provided equal protection and tetracycline was slightly less effective. However, when given in the feed the order of efficacy was chlorotetracycline, oxytetracycline, and tetracycline (Shelton and Olson 75). In experimental field trials 100 gm. provided complete and 50 gm. per ton of chlorotetracycline given continuously provided satisfactory control of IS. Birds exposed at three weeks of age were more severely affected than those exposed at five weeks. Chlorotetracycline was less effective when the culture inoculated was more pathogenic (Olson and Shelton 76). The efficacy of either chlorotetracycline or furazolidone (nf-180) was related to the pathogenicity of the agent with chlorotetracycline being approximately four times as effective as nf-180 (Olson and Shelton 77). These results were not in agreement with those of Cosgrove (78) who reported complete protection with 100 gm. of nf-180 per ton of feed and 80 percent protection with 50 gm. of nf-180 when given seven days before inoculation to birds experimentally inoculated with IS.
Shelton and Olson (79) found single injections of 25 to 200 mg./lb. of body weight of aqueous dihydrostreptomycin alone and the aqueous plus 100 mg. in oil controlled IS if given at the time of experimental inoculation but did not control the swellings caused by IS if given four, eight or 12 days after experimental inoculation. Slight improvement was noted when the higher levels were used.

**PARASITIC DISEASES**

*Coccidiosis.* Rubin *et al.* (80) found Nicarbazin to be highly effective as a prophylactic drug both at the recommended level of 0.0125 percent and also at a level of 0.0625 percent.

Berg *et al.* (81) studied Nitrofurazone and Nicarbazin as growth stimulants and coccidiostatic agents for young chicks. This investigator found that Nicarbazin at a level of 0.0125 percent resulted in no increase in growth rate, decrease in feed efficiency, freedom from coccidial infection, development of immunity to *E. tenella*, and lack of development of immunity to *E. necatrix*. A level of 0.02 percent of the same drug depressed the growth rate. Nitrofurazone at levels of 0.0055, 0.0070 and 0.0083 percent had no effect on growth, improved feed efficiency, did not afford complete protection from coccidial infection, permitted development of immunity to *E. tenella*, and permitted development of immunity to *E. necatrix* in over 50 percent of the birds.

Biological studies on the anticoccidial agent Nicarbazin were conducted by Ott (82) who stated that the chemical complex of two compounds has at least 10 times the anticoccidial potency of the active component. Tolerance studies showed that continuous feeding of Nicarbazin permitted optimal growth and feed utilization in young chicks and did not interfere with sexual maturity, egg production or fertility in adult chickens. However, in New Hampshire hens egg-shell pigmentation was greatly decreased and hatchability was reduced. Both returned to normal after discontinuance of the medicated feed.

Soluble Furacin-mix in drinking water at 0.008 percent was found to be a valuable agent in preventing or reducing mortality in outbreaks of cecal coccidiosis by Johnson (83). Arundel and Sutherland (84) found Nitrofurazone and sodium sulphadimidine effective in preventing death from a coccidial infection that killed 15 out of 30 untreated chickens.

It was determined by Shumard (85) that a dosage of 0.0008 percent of water-soluble Furacin (Eaton) was the most effective dose of the drug against artificially induced infection with *Eimeria necatrix*. The drug was found not to interfere with the development of immunity to this species of coccidium.

Cuckler and Malanga (86) reported that the feeding of Nicarbazin significantly reduced the damaging pathological effect of avian coccidiosis but did not interfere with development of acquired immunity. Experiments having to do with the effect of antibiotics on avian coccidiosis and enterohepatitis (blackhead) were also reported by Cuckler and Malanga (87). Spiramycin and chlortetracycline had the same order of activity against cecal
coccidiosis as sulfathiazole. None of the antibiotics tested had significant activity in experimental enterohepatitis in turkeys.

Davies (88) investigated the life cycle of *E. necatrix* and found oocyst production can commence on the sixth day after infection and sporulation can take place in a minimum period of 21 hours. Treatment with 0.4 percent sulphasalazine combined with 0.02 percent pyrimethamine was found to be a highly effective synergistic combination in combatting the disease.

Becker et al. (89) presented statistical data on the average sizes (length x width) with average standard deviation and average shape—indexes of six species of *Eimeria* including *E. maxima, E. brunetti, E. tenella, E. necatrix, E. acervulina,* and *E. mitis.* Measurements on *E. maxima, E. brunetti,* and *E. tenella* are not in actual disagreement with data of previous workers.

Uricchio (90) studied the therapeutic effect of allyl acetone in young chicks infected with *Eimeria tenella;* the chemical did not prove sufficiently effective to be useful on a practical basis. Clarkson (91) reported the successful experimental infection of turkey poults with *Eimeria adenoides* isolated from a natural case in Great Britain.

**Ascariasis.** It was determined by Riedel and West (92) that supplemental phenylalanine in the diet was not instrumental in increasing resistance to ascariasis when New Hampshire chicks were artificially exposed by administration of 500 embryonated ova per bird of *Ascaridia galli.* Shumard (93) reported that laboratory trials with piperazine citrate indicated high anthelmintic efficiency against *A. galli* when between 2,000 and 4,000 mg. per gallon of drinking water is administered. Preventive levels as low as 1,000 mg. per gal. were highly effective in preventing establishment of this parasite.

Moran and Mizelle (94) reported that daily examination of chicks inoculated with 800 infective eggs of *Ascaridia galli* revealed the posterior third of the intestine to be a significant and heretofore unrecognized habitat of the parasite. A total of 99.6 percent of infecting larvae remained in the layer of mucus adherent to the intestinal wall after the gut was flushed with water under pressure. Infective eggs hatched in the anterior third of the intestine as early as 30 minutes after inoculation.

Kerr and Walde (95) studied the anthelmintic activity of tetravalent tin compounds. It was found that compounds with the formula R₂SnX₂ showed the most consistent activity against both *Ascaridia galli* and *Raillietina cesticiulus.*

**Histomoniasis.** Costello and DeVolt (96) found that Furoxone (Furazolidone, Eaton Laboratories) incorporated in mash in concentrations of 0.011, 0.0167 and 0.033 percent suppressed clinical infection with enterohepatitis (blackhead) in turkeys but, especially in lowest concentration studied, failed to prevent formation of slight lesions in some cases. The transmission of histomoniasis in turkeys was studied by Lund (97) who obtained data showing that up to 20 percent of birds ingesting unprotected histomonads exhibited pathological changes. Survival of the protozoan parasite *Histomonas meleagridis* in feces of infected birds was studied by Farr (98) who was able to recover *Heterakis* larvae and histomonads at autopsy.
from birds fed droppings that had remained on soil from 17.5 to 66 weeks. Nithiazide [1-ethyl-3-(5-nitro-2-thiazole) urea] was reported to be more potent and less toxic than 2-amino-5-nitrothiazole when fed to turkeys with enterohapatitis in experiments conducted by Cuckler and Malanga (99). The drug was most effective when administered prior to infection or beginning three to seven days after infection.

Horton-Smith and Long (100) studied infection of chickens (Gallus gallo-lus) with histomonad suspensions and reported that the disease could be transmitted only when chickens were starved or had received an alkali mixture immediately before administration of the parasites.

**Taeniasis.** Efficacy of several compounds causing elimination of tapeworms from laboratory-infected chickens was investigated by Edgar and Teer (101). A single dose of 15 mg. of di-n-butyl tin (DBT) oxide, 37.5 DBT maleate, dibenzyl tin dichloride, or 75 mg. of DBT sulfide, usually resulted in 95 to 100 percent elimination of R. cesticullus and C. infundibulum. Polystat fed continuously at the rate of four pounds per ton of feed was effective in preventing the development of Ascaridia galli and C. infundibulum. Abdou (102) found di-n-butyl tin dilaurate to possess anthelmintic value against Davania proglottina. Drug was administered in gelatin capsules and the effective experimental doses varied from 1.0 ml. to 0.5 ml. per kilo. of body weight.

**Leucocytozoon Infection.** The histopathology of Leucocytozoon simondi in two groups of ducks was studied by Newberne (103). Significant lesions found in birds with fatal infection were liver necrosis and marked enlargement of the spleen. In experiments with the same parasite Cowan (104) presented evidence of reactions against the parasite including possible encapsulation and destruction by phagocytes.

O'Meara (105) reported that blood smear sampling from wild flock and Wood Ducks of eastern Maine indicated infections of Leucocytozoa, Haemoproteus and microfilarial forms. This reservoir of infection is suggested as of possible importance to duck raising in Maine. Parasitism of adult turkeys in Florida by Leucocytozoon smithi was reported by Simpson et al. (106). Infection occurred simultaneously with fowl cholera in one instance and with visceral leukosis in a second. The possibility that Leucocytozoon infection acts as a stress factor increasing the severity of coexisting infections was postulated. Similium slossonae breeding near turkey ranges was considered as a probable vector for L. smithi in Florida.

**Malaria.** McGheen (107) tested the susceptibility of five species of birds and three mammals to four species of avian malaria. All birds' cells were susceptible to all species of avian malaria but not to the same degree. Bishop (108) found no potentiation between pyrimethamine and quinine when the activity of these drugs was tested against Plasmodium gallinaceum. Bishop and McConnachie (109) also studied factors affecting emergence of gametocytes of P. gallinaceum from erythrocytes and exflagellation of male gametocytes.
TRANSMISSIBLE DISEASES OF POULTRY

Rao and Sirsi (110) studied the relationship of B complex vitamins to avian malaria and stated that vitamins play an important role in host-parasite relationship. Effects of vitamin deficiency and supplementation vary with different kinds of infections. Thiamine depletion adversely affects multiplication of the malarial parasites. McGhee (111) was unable to produce discernible infection of chick embryos with canary blood infected with Plasmodium cathermerium. Inoculation of the same quantity of parasites into duck embryos resulted in an initial low grade infection gradually increasing over a period of several days. Becker et al. (112) reported naturally occurring Plasmodium and Haemoproteus infection in the common pigeon in Iowa.

Trichomoniasis. Trichomonas gallinarum infection was reported in chukar partridges (Lectoris graeca) by Wichmann and Bankowski (113) who examined five young birds from a flock of 35, most of the other 30 partridges having died during the previous week. Circumscribed, pinkish-yellow, flat, spherically irregular liver lesions varying in size from 0.5 to three cm. were observed. Ceca were filled in some instances with yellow caseous cores. The effect of Furazolidone (Eaton) as a therapeutic agent against pigeon trichomoniasis was studied by Stabler (114). The drug appeared too toxic at effective therapeutic levels to be employed against this parasite. Doses of 35 to 200 mg. per day per bird were studied. A dose of 10 mg. per bird per day for seven days was nontoxic but only two out of 10 birds became free from T. gallinae.

Hexamitiasis. Vance and Bigland (115) described the symptoms, pathology and control of 15 outbreaks of hexamitiasis in turkey flocks of Alberta, Canada. Symptoms were observed to be closely related to age of affected birds. Young birds may fail to exhibit signs of illness before death. In addition to ruffled feather and huddling near heat, affected birds commonly void foamy, brown droppings. Serious loss of flesh often followed without reduction in feed consumption. The most characteristic changes observed at autopsy were in the middle portion of the intestine which was atonic and thin-walled to a degree of transparency in many cases. The contents were mucoid and watery. Hexamita could be demonstrated only in specimens submitted alive. Aureomycin has been recommended as an effective agent against experimental infection. Atabrene and di-n-butyl tin dilaurate appeared not to be effective. The administration of 2-amino-5-nitrothiazole was partially successful.

The life cycle and seasonal transmission of Ornithofilaria fallisensis (Anderson) in domestic and wild ducks was extensively reported by Anderson (116). White Pekin ducks kept in a park were found to be infected during the black fly seasons of 1952 to 1955. Since parasite-free adult ducks became infected during exposure, it was concluded infected birds acquire immunity to O. fallisensis. The Black Duck (Anas rubripes) was suggested as a reservoir of infection in the park.

Spirochetosis. A case of fowl spirochetosis in New Mexico was reported by Francis (117). Bloody mucus from the upper respiratory passages of S. C.
White Leghorn pullets 14 months of age yielded a spirochete when stained with Gram's stain. The spleen exhibited typical swelling and mottled appearance.

MISCELLANEOUS DISEASES OR CONDITIONS

*Quail Bronchitis.* A virus was isolated from a case of quail bronchitis in Texas by DuBose *et al.* (118). The virus was closely related or identical with the virus Olson isolated in 1950 from quail bronchitis in West Virginia.

*Pendulous Crop in Turkeys.* The effect of diet on the incidence of pendulous crop was studied (119) and it was found that pendulous crop could be produced by feeding glucose monohydrate, which in turn apparently made conditions much more favorable for a tremendous increase in yeast cells in the crop.

*Cataracts.* A new flock problem was described in Texas (120) involving 18 flocks with an average of five percent of the birds involved. The gross lesions were opacity of the lens. The cause was not determined.

*Inapparent Virus Disease of Chickens.* Yates (121) isolated seven strains of a viral agent (celo) that produced consistent embryo lesions and death patterns. The celo virus was not found to be related to known viruses. Neutralizing antibodies were found in the serum of chickens from many areas.

RECOMMENDATIONS

The Committee recommends that the United States Livestock Sanitary Association take action to change the present regulations which prevent the Pure Food and Drug Administration from divulging data on the toxicity of new drugs. The situation at the present time is that such information cannot be released by the Food and Drug Administration even though it is pertinent to the use of the drug. The result has been that toxic effects of a particular drug must await investigation by college and experiment station workers. The information on toxicity of the drug should be released at the same time the drug is approved for sale.

It is also recommended that this action be applied to the Biological Products Licensing Section of the Agricultural Research Service regarding the release of information on new biological products at the time they are approved for sale.

REFERENCES


79. Shelton, D. C., and Olson, N. O.: Control of infectious synovitis. 9. The efficacy of dihydrostreptomycin sulfate as related to the time of experimental infection. 5th An. Sympos. on Antibiotics. 1957.


REPORT OF THE COMMITTEE ON PUBLIC HEALTH

A. L. Brueckner, Chairman, Baltimore, Maryland; C. D. Carpenter, Chicago, Illinois; G. H. Good, Cheyenne, Wyoming; H. J. Rollins, Raleigh, N.C.; J. Steele, Atlanta, Georgia; O. Sussman, Princeton, New Jersey; F. P. Wilcox, Los Angeles, California.

Your Committee feels that a sound, forward step was taken during the past year when the Congress enacted legislation requiring inspection for wholesomeness of all poultry and poultry products entering interstate commerce. This legislation embodies standards of sanitation for buildings, equipment and processes, as well as bird by bird post-mortem inspection for wholesomeness. As this report is being written, the United States Department of Agriculture is holding 12 regional meetings on proposals for regulations under the law, and it is this Committee's recommendation that such regulations, when and as written, be considered by this organization for adoption, as representing national standards in this important field of wholesomeness of product and for sanitary practices.

The question naturally arises as to whether the responsibility for administering this law belongs in the Agricultural Marketing Service or in the Meat Inspection Division of the Agricultural Research Service. Your Committee feels that future developments will decide this matter.

PSITTACOSIS

Psittacosis-ornithosis continued to appear throughout the country, although the number of cases reported during the first six months of 1957 was considerably less than for a like period of the previous year. Only one epizootic occurred, this in turkeys in Oregon where there had been a severe epidemic the year previously. This disease was recognized in turkeys in Vancouver under conditions similar to those encountered in the Oregon episode. The epizootic is now under investigation by Canadian authorities.

The disease has occurred sporadically in humans throughout the country and the majority of these cases resulted from exposure to psittacine birds. The severity of the disease in humans is kept to a minimum when adequate antibiotics are given early in the course of the disease.

Procedures for the control of this disease in avian species are being tested in many parts of the country. The use of antibiotic impregnated feed under controlled conditions appears to be effective in eliminating infection. Only feed prepared under laboratory conditions has been used. Efforts to produce large quantities of antibiotics treated bird feed commercially have not proven entirely satisfactory. Studies are continuing. Treatment of infected turkey flocks with antibiotic feed has not proved as successful as has treatment in psittacine birds. Results have been variable. We recommend the continuation of research to develop a method of treating poultry for ornithosis infection with antibiotics in feed.
TUBERCULOSIS

Bovine tuberculosis is causing concern to many livestock sanitary officials because of the occurrence of severe outbreaks in herds of cattle previously free of this disease for many years. The Livestock Disease Control Division of the Michigan Department of Agriculture and the Animal Disease Eradication Branch of the United States Department of Agriculture published a Progress Report on the Bovine Tuberculosis Eradication Program as of December 1, 1956.

The Michigan report indicates what complacency can lead to. They point out that there has been a notable increase of bovine tuberculosis in recent years. The Michigan authorities estimate that their program has slid back to the same level of infection that existed in 1929.

In light of the reported incidence of bovine tuberculosis in cattle in Michigan and other midwestern states, it behooves the Public Health Committee of the United States Livestock Sanitary Association to investigate this problem and determine if there should be more stringent requirements on dairy cattle. Your Committee feels that further complacency in this area could be disastrous and suggests that dairy herds in areas where infection rates exceed the national rate be tested annually until their rate has declined to the national figure.

LEPTOSPIROSIS

In recent years there has been an increase in reported human cases of leptospirosis in the United States. Molner et al. (J.A.M.A. 136: 814-819, 1948) summarized the reported cases from 1905 to 1948 according to geographic location. During this 43-year period there were 220 cases in 24 states, eight cases in which location was not known, and an additional 78 cases studied by these authors in Michigan. In contrast, a summary of cases detected through serological tests in the CDC laboratories during a four-year period (1953-1956) plus those reported to the Communicable Disease Center by State health departments or to the National Office of Vital Statistics revealed a total of 392 in 39 states. Information was obtained at CDC concerning the probable source of 64 cases which occurred during the past three years. Of these, 25 (39 percent) had had contact with infected cattle or swine in abattoirs or on farms; 17 (26 percent) had been drinking, swimming, or had been accidentally immersed in presumable contaminated water; 12 (19 percent) had had contact with dogs in their homes or in veterinary hospitals; seven (11 percent) had been exposed to rats; two (three percent), to wild animals and one (two percent), to a goat.

The fact that the probable source of more than one-third of these recent cases was found to be contact with infected cattle or swine may be attributed, in part, to the rapid spread of bovine leptospirosis in the United States. York (Proc. United States Livestock Sanitary Association, 295-300, 1951) reported in 1951 that leptospirosis had occurred in 12 states. A summary of animal morbidity reports to the Communicable Disease Center, published
reports, and positive serum samples examined by CDC laboratories revealed the occurrence in 42 states in 1956. Agricultural Research Service (United States Department of Agriculture, Estimated Losses in Agriculture, 129, 1954) estimated that animal losses from bovine leptospirosis were over 112 million dollars.

The attention of veterinary and medical laboratories should be directed toward detection of infection in domestic animals and humans, not only with the more common serotypes, *L. canicola*, *L. icterohaemorrhagiae*, and *L. pomona*, but with other serotypes as well. Recent studies on wild mammals in southwest Georgia (McKeever, *et al.* In Manuscript) have revealed the presence of a variety of leptospiral serotypes hitherto unrecognized in the United States in raccoons, opossums, and skunks. These types include *L. australia A*, two serotypes that belong to the hyos serogroup (Galton *et al.*, Pub. Health Rep. 72: 431-435, 1957), *L. grippotyphosa*, and a member of the hebdomadis serogroup. In addition, the raccoon was detected as the first animal host of *L. autumnalis* in the United States. (McKeever *et al.* In Manuscript). Further study of these wild mammal hosts is indicated to determine the prevalence of infection in other areas of the country, the duration of the carrier state, and their role in the epidemiology of leptospiral infection in humans and domestic animals.

**TULAREMIA**

Tularemia presents human disease problems in some western states by direct human contacts with diseased animals and by insect vectors. In order to combat this situation effectively, close relationship must be maintained among livestock sanitary officials, health officials, practicing veterinarians and physicians.

**RABIES**

Closer contacts between veterinary officials concerned with rabies control and health departments, and vice versa, are essential in the control of this disease. Where the control activity is the responsibility of the health department, the livestock sanitary official should be kept informed so that he may properly handle requests for the shipment of animals and for the protection of domestic animals. Obviously, cases of rabies known to the livestock sanitary official should be immediately passed on to the health department for the protection of exposed humans.
RABIES CONTROL IN ST. LOUIS CITY—SIX AND ONE-HALF YEARS AFTER ITS LARGEST EPIZOOTIC

KENNETH V. SHASHEK, D.V.M., M.P.H., Public Health Veterinarian; J. EARL SMITH, M.D., Health Commissioner, St. Louis Health Division.

The City of St. Louis, Missouri, experienced its largest epizootic of canine rabies—326 cases—during the year 1951.

The purpose of this paper is to evaluate the rabies control program of the St. Louis Health Division six and one-half years later. During that interval of time many changes have been effectuated which have definitely improved the administration of our rabies control program. We propose to present a chronological history of the program since 1951 in the hope that other communities might benefit from our experience.

CASES OF LABORATORY CONFIRMED RABIES

ST. LOUIS, MO, 1922-1957 (NOK)

The geography of St. Louis city can best be described as a half-moon along the Mississippi River completely surrounded on three sides by St. Louis County. Across the river lie Madison and St. Clair Counties in Illinois. The major municipality just east of the city is East St. Louis, Illinois.

Public health jurisdiction in the St. Louis Metropolitan Area includes three major official agencies: The St. Louis Health Division of the City and the St. Louis County Health Department, in Missouri, and the East Side Health District for East St. Louis and three adjoining townships in Illinois.
Rabies has become an increasingly important and complex public health problem in the city. Six rabies epizootics have occurred, with each outbreak becoming more serious and of longer duration than the previous. From 1936 to 1950 there was an average of 105 rabid animals each year, mostly in dogs (1).

Because we were confronted by a full blown epizootic in May, 1951, steps were taken to organize a rabies inoculation program in which Health Division personnel, civic volunteers, and members of the Greater St. Louis Veterinary Medical Association participated. Eighteen immunization clinics located at strategic points throughout the city were set up and operated four hours
daily for seven days. Thirty-eight thousand dogs were vaccinated in these clinics. In addition approximately 20,000 were inoculated in private offices of veterinarians (2).

During the first six months of 1951 some 58,000 dogs were immunized against rabies in the city. This was estimated to be 70 percent of the dog population. The prevalence of rabies dropped sharply following the intensified vaccination program (3).

In the adjacent health jurisdictions of St. Louis County and the East Side Health District, the disease continued at a relatively high rate for the remainder of the year. Control methods utilized some sporadic vaccination clinics involving small numbers of animals over an extended period (4).

In St. Louis City, beginning in 1952 and extending through 1956, there has been a total of 106 cases of rabies with a mean of 21.2 for the period. Rabies has not been reported in bats or other wild carnivora. No cases of rabies in any species, either clinical or laboratory confirmed, have been reported up to the present time this year.

During the corresponding period (1952 through 1956) St. Louis County reported an incidence of 255 cases and the East Side Health District 273 cases. The averages for these jurisdictions are 51.0 and 54.6 respectively.

In 1952 the East Side Health District experienced an epizootic of rabies in which 175 cases were reported. So far in 1957 the incidence of rabies in all three health jurisdictions is very low—none in St. Louis City, eight in St. Louis County, and one in the East Side Health District.
Rabies Control Under the Health Division

Prior to the 1951 epizootic the St. Louis Municipal Dog Pound was a responsibility of the City Marshal. In 1952 a rabies ordinance was passed by the Board of Aldermen requiring annual vaccination before licensing; however the dog pound continued under the jurisdiction of the City Marshal. The Health Division had no control over the dog pound and the dog population.

During 1953 a grand jury investigation revealed various irregularities in the operation of the Municipal Dog Pound. As a result of their recommendations, the Mayor ordered the Health Division to operate the pound pending passage of a new ordinance.

In March, 1954, the Board of Aldermen passed an ordinance which gave the Health Division complete authority over the rabies control program.

At long last, rabies control became a part of the communicable disease control activities under the direction of a public health veterinarian. Restraint upon the dog population was, and is still, maintained by continuation of the rabies quarantine, which is in effect a leash law.

In addition to the impounding of stray dogs, the ordinance requires an 11-day observation period of all biting dogs reported. Through rules and regulations, the Health Commissioner has permitted licensed biting dogs to be held in bonded veterinary hospitals. Unlicensed and unimmunized dogs must be observed in the isolation unit of the pound. The public health veterinarian is responsible for coordinating all facets of the Rabies Control Program with the Pasteur Clinic where practically all animal bite patients are referred.

Reorganization of the Municipal Dog Pound by the Health Division presented many problems. Some of the more pertinent of these were:

1. All personnel were placed under civil service.
2. Through neglect over the years, extensive building repairs were necessitated.
3. A Health Division accountant assigned from another section organized records, forms, and a system of auditing both animals and cash receipts.
4. Fees for services were established to make the program partially self sustaining.
5. Modern sanitation practices based on industrial mechanized methods were instituted.
6. A small dispensary was organized to give medical care to impounded animals and aid in fulfilling vaccination requirements.
7. Various forms of public relations were initiated in order to help reduce community hostility toward the Municipal Dog Pound. Operation of the dispensary for impounded animals, notification of owners of dogs found stray, answering complaints, and the welcoming of visitors are examples.
The impounding of stray and biting dogs has averaged 4,655 animals annually for a three-year period of operation under Health Division supervision. About 40 percent of all impounded stray dogs are claimed. Our experience has shown that 63 percent of all impounded dogs have not been vaccinated for rabies. The dog pound also has collected 21 percent of all dog licenses sold in the city.

Education is an important function of any rabies control program. In St. Louis all animal bites are reportable to the Police Department for investigation. In order for police officers to fully appreciate this responsibility, it has been necessary for the public health veterinarian to set up an educational program for all recruits of the police department. Lectures supplemented with audio-visual aids are used in the police academy.

Educational media is directed to school children, service and fraternal groups, and to the general public. Leaflets and posters have been distributed in schools, at the Dog Pound, in health centers, and in the Pasteur Clinic. Interested groups may procure a speaker and film on community rabies control. The Health Commissioner’s weekly bulletin conveys several rabies messages throughout the year.

In March, 1957, the St. Louis Health Division and the St. Louis County Health Department, in cooperation with the Greater St. Louis Veterinary Medical Association, sponsored a Rabies Control Week. In the city, 15 clinics were operated four hours daily for four evenings. This was the first attempt to utilize mass vaccination without the urgency of an epizootic. During this week 4,300 dogs were vaccinated in the city and 4,700 in the county. Response, at first, was slow but increased coverage by the press, radio, and television helped stimulate clinic attendance. Weather and light conditions due to the early spring date were not conducive to great public response.

DISCUSSION

There is no question that mass immunization proved its effectiveness during the epizootic of 1951. After this episode the lawmaking body of the city demonstrated its faith in compulsory annual vaccination and in rabies control by placing this function under Health Division supervision. The control of rabies is a preventive medicine service in a public health agency. Such authority cannot be effectively delegated to private organizations (5).

The public is now becoming more rabies conscious during non-epizootic years. The veterinarians of the Greater St. Louis Veterinary Medical Association through an active rabies committee, have recognized the public health aspects of a sound rabies control program and have made substantial professional contributions to a serious community health problem.

The increased use of chick embryo rabies vaccine throughout the dog population, especially at the early age of three months rather than waiting to maturity, has strengthened the biological barrier around the disease (6). When traditional control procedures prove difficult, the use of such an auxiliary tool assumes a key position in the eradication of urban rabies (7).
The intention of the St. Louis ordinance requiring annual rabies vaccination cannot be questioned. Unfortunately it has included the traditional use of the dog license, which has failed to fulfill the very important task of providing an index of the dog population. This is demonstrated by the normal issuance of 10,000 or less dog licenses annually when it is estimated that there are 85,000 dogs in the city. The dual requirement of vaccination and license may discourage dog owners from complying with the law. The issuance of dog licenses may actually cost the city more than realized in revenue and may antagonize owners of dogs (8). The substitution of a registration system based on rabies vaccinations to establish a dog census would lend itself as a means of education and providing a population figure (9). Such a system would have the appropriate effect of an organized and properly functioning public health program.

**SUMMARY**

1. The incidence of rabies in St. Louis City and the surrounding metropolitan area since the 1951 epizootic has been described.

2. The Health Division has operated the rabies control program utilizing compulsory rabies vaccination, licensing, and operation of the Municipal Dog Pound for three and one-half years.

3. Education has become an important part of the municipal rabies program.

4. The effect of licensing as a requirement of dog ownership has been questioned.

**REFERENCES**


2. Ibid.

3. Ibid.

4. Ibid.


REPORT OF COMMITTEE ON RABIES

Vernon D. Chadwick, Chairman, Jackson, Mississippi; H. R. Cox, Pearl River, New York; D. L. Haley, Albany, New York; H. A. Milo, Harrisburg, Pennsylvania; E. S. Tierkel, Atlanta, Georgia.

RABIES MORBIDITY TRENDS - 10 YEARS - 1946 - 1955

It has been during the past 10 years that practically all of the present veterinary public health programs within State health departments have been inaugurated and developed. Rabies control has been the principal zoonoses activity of most of these programs. The CDC rabies program of research, technical consultation and training was developed during this same 10-year period.

**TABLE I**

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Wildlife</th>
<th>Cats</th>
<th>Livestock</th>
<th>Man</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>1946</td>
<td>8,384</td>
<td>956</td>
<td>455</td>
<td>1,055</td>
<td>22</td>
<td>10,872</td>
</tr>
<tr>
<td>1955</td>
<td>2,657</td>
<td>1,915</td>
<td>343</td>
<td>924</td>
<td>5</td>
<td>5,844</td>
</tr>
</tbody>
</table>

Progress achieved in the overall national incidence of rabies during the past decade is reflected in the annual reported cases in Table I. The total number of cases in 1946 was 10,872 which declined steadily to 5,844 cases for 1955, a decrease of one-half. By the same token the human cases dropped from 22 in 1946 to five in 1955, a decrease of 77 percent. Although the dog still accounts for most of the cases and remains the most important vector of the disease in most parts of the country the striking decline in canine cases from 8,384 in 1946 to 2,657 in 1955 (68 percent) is eloquent testimony to our control efforts over the past 10 years. In sharp contrast to the steady recession of the canine disease is the increase of reported cases in wildlife from 956 to 1,915 in 1955. Thus a nutshell evaluation of our progress indicates a need for consolidating our gains by intensifying the campaign against the disease in the canine vector and expanding our investigational activity in the epidemiology of the disease in sylvatic vectors.

RABIES MORBIDITY TRENDS FIRST SIX MONTHS - 1957

Incidence of rabies in the United States for the first six months of 1957 according to reports received by the United States Public Health Service, totaled 2,550. This represents a decline of 272 cases compared with the same period of 1956 and a decline of 1,532 compared with the same six-month period of a five-year median during 1952 to 1956 inclusive. Thus far in 1957 three human cases have been reported: one in California, one in South Carolina, and one in a resident of Tennessee who was exposed in Arkansas.
The number of annual reported cases of rabies continued its decline in 1956. The total number of cases (provisional) reported to the Public Health Service in the NOVS weekly telegraphic reports was 5,124, a drop of 729 cases from the 1955 figure as reported on the annual United States Department of Agriculture questionnaire to the States. The decrease was fairly uniform throughout the country with rather marked declines in California, North Carolina, New York and Georgia. A substantial rise in incidence occurred in Louisiana with smaller increases in Indiana and South Carolina.

The bat rabies tally stands at approximately 150 isolations from 14 species of insectivorous bats from 1953 to the end of 1956. Bat rabies have thus far been reported from 15 states with preponderance of cases from large-scale surveys in the southwest and southeast. The States reporting bat rabies are Florida, Pennsylvania, Georgia, Alabama, New York, Texas, Oklahoma, Utah, California, Montana, Ohio, Michigan, Louisiana, New Mexico and Minnesota.

The number of human rabies deaths jumped from five cases in 1955 to nine cases in 1956. Almost half (four cases) of these occurred in Dallas, Texas. Seven of the cases were attributable to the bite of a dog, one to a cat and one (the case of Mr. George Menzies) to working with rabid bats. Vaccine treatment was not given in seven of the cases. Of the remaining two cases, incomplete vaccine therapy was administered; in one (Dallas, Texas), where the incubation period proved extremely short (16 days), eight daily doses of vaccine was started nine days after exposure, and in the other case (Mobile, Alabama) seven daily doses were administered and then discontinued against advice of the physician. Four of the deaths occurred in children under 15 years of age, three in persons over 60 years and the remaining two were 26 and 41 years.
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<th>Locality</th>
<th>Date of Report</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Post-Exposure Prophylactic Treatment</th>
<th>Biting Animal</th>
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<td>1/4/56</td>
<td>26</td>
<td>F</td>
<td>Bitten on right thumb</td>
<td>9 months</td>
<td>Unknown</td>
<td>7 daily stopped after 7</td>
<td>Dog</td>
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<td>Lake Co., Indiana</td>
<td>4/21/56</td>
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<td>Bitten on finger</td>
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<td>4 days</td>
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<td>4/26/56</td>
<td>7</td>
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<td>Unknown</td>
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<td>72 hours</td>
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<td>12</td>
<td>M</td>
<td>Bite</td>
<td>3 weeks</td>
<td>1 day</td>
<td>None</td>
<td>Dog (?)</td>
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<td>2/14/56</td>
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<td>3 days</td>
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<td>F</td>
<td>Bitten on lip</td>
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<td>9 days</td>
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<td>M</td>
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<td>Bitten on leg</td>
<td>7 weeks</td>
<td>9 days</td>
<td>None</td>
<td>Cat</td>
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</table>

United States Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Atlanta, Georgia.
RABIES REPORT

The Third Report (1957) of the WHO Expert Committee on Rabies (Technical report Series No. 121) has now been published.

The new report contains a revised guide for specific human post-exposure treatment with explanatory notes. This report follows the pattern of the previous reports and gives more current information on the use of vaccines and serums. Newer evidence is presented showing the efficacy of local infiltration of hyperimmune serum beneath the bite wound. A new section has been added on human pre-exposure prophylactic vaccination of high risk occupational groups such as veterinarians, laboratory workers, dog wardens, etc. The annexes include suggested forms for case records of human rabies exposures and for international health and vaccination certificates for dogs and cats. Recommendations for immunization of animals are strengthened by field corroboration of earlier laboratory experiments.


* * * * * * * * * * *

An important study in comparative routes of administration with chick embryo rabies vaccine in puppies was reported by R. V. Johnston, et al., in the Journal of the A.V.M.A., 130:2, January 15, 1957. In this experiment, nine out of 10 (90 percent) of five-month-old pups vaccinated intramuscularly resisted infection nine months after vaccination with a challenge dose which killed nine out of 11 (82 percent) of the non-vaccinated controls. Only six of 17 (35 percent) of those vaccinated subcutaneously resisted infection four and three-quarter months after vaccination with a challenge dose which killed all three non-vaccinated controls. This study points out the importance of intramuscular inoculation of dogs with chicken-embryo modified living virus rabies vaccines.
TABLE 3
Reported Incidence of Rabies in the United States 1938-1956

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<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>
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Totals 118,733 12,328 620 719 914 7,228 172 17,160 444 158,318
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**TABLE 4**

*Rabies Reported in the United States During Calendar Year 1956*
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<th>Dogs</th>
<th>Cats</th>
<th>Cattle</th>
<th>Horses and Mules</th>
<th>Sheep</th>
<th>Swine</th>
<th>Goats</th>
<th>Foxes</th>
<th>Skunks</th>
<th>Raccoons</th>
<th>Squirrels</th>
<th>Opossums</th>
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<th>Mole-Rats</th>
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### Bat Rabies

Since the country’s first reported case of bat rabies occurred in Florida in June 1953, there have been 175 cases diagnosed in widely diverse geographical areas of the United States. Rabies in bats has been reported from Florida, Pennsylvania, Texas, California, Montana, Ohio, Louisiana, New Mexico, Georgia, Alabama, Utah, Oklahoma, Minnesota, New York, and Michigan. Four species of tree living or solitary bats, and nine species of colonial or cave dwelling bats have been implicated thus far. All are the insectivorous variety. The greatest number of rabies virus isolations have been from the Mexican freetail bat (*Tadarida Mexicana*) in southwestern United States.

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**Rabies**
### TABLE 5

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Your Committee on Rabies recommends:

(1) The need for consolidating our gains by intensifying the campaign against the disease in the canine vector and expanding our investigational activity in the epidemiology of the disease in sylvatic vectors.

(2) Carry out the enforcement of present laws, rules and regulations throughout the country for better control of rabies with eventual eradication in mind.
OBSERVATIONS ON BLUETONGUE IN SHEEP

B. R. McCrory, D.V.M., M.S.; R. C. Bay, B.S., D.V.M., and
N. M. Foster, B. S.

Denver, Colorado

It is not certain when bluetongue, an exotic, transmissible, viral disease of sheep, first appeared in the United States. A disease of sheep, which in retrospect is suggestive of bluetongue disease, was reported (1) from southern Arizona in 1947. Losses were severe, 800 out of 5,000 sheep, and a less severe outbreak followed in 1949. In 1954 bluetongue was diagnosed in this same area by the Denver station. A disease called "soremuzzle" of sheep which occurred in 1948 and 1951 in Texas was reported by Hardy and Price (2) in 1952. They suggested the possibility that the disease might be bluetongue. In 1953, McGowan (3) reported the appearance of a disease in 1952 resembling soremuzzle or bluetongue in California sheep. The first isolation of bluetongue virus in the United States was made by McKercher et al. (4) in 1952 from sheep in California.

From the Animal Disease Research Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Denver, Colo.

Soon after the presence of bluetongue virus in the United States was established, a protective vaccine (5) was developed which was similar to the quadrivalent modified live-virus vaccine that was being used successfully as an immunizing agent in South Africa (6). It had already been demonstrated that several immunologically different strains of bluetongue virus were present in South Africa. Because of this, both of the two American isolates which were then available, California BT-8 and California BT-11, were incorporated in the vaccine (6) prepared for use in this country. Cross-immunity tests, however, subsequently showed these two isolates to be immunologically identical (7).

The severity of the outbreak of bluetongue in California and Texas in 1952 was the primary factor leading to the establishment of a bluetongue virus laboratory by the Animal Disease and Parasite Research Division at Denver, Colorado. The over-all plan of the work was to make virus isolations, to search for different antigenic virus types, to confirm the field diagnosis of clinical bluetongue, and to conduct other studies on the virus.

The facilities consist of a separate laboratory; eight isolation rooms, each of which will house six to eight exposed sheep; and eight small buildings, which will house a total of 40 immune sheep. In addition there are corrals for holding susceptible sheep which are to be used for test purposes. Supporting units such as personnel facilities, a building for small animals, and an incinerator are provided.
Because bluetongue is reported (8, 9) to be transmitted by an insect, *Culicoides*, it was necessary to make the isolation rooms insectproof and to maintain a program of strict sanitation in order to confine the virus. That this has been successfully accomplished is demonstrated by the fact that in no instance have in-contact temperature-control animals become infected and that susceptible sheep have been kept within 75 feet of the infected quarters for two and one-half years without accidental spread of the infection.

Blood samples from 132 flocks of sheep suspected of having bluetongue have been received at the Denver laboratory for confirmation of the clinical diagnosis. With few exceptions the blood samples that have been submitted to the laboratory or obtained directly by us have been collected in an equal volume of OCG* preservative solution and stored at 5°C. All the blood specimens from field cases, most of which were submitted by the Animal Disease Eradication Division, were accompanied by a questionnaire giving the flock history, together with the symptoms and lesions shown by the animals bled. The 132 flocks represented 75,810 sheep. An analysis of the reports on 72,015 of the sheep showed the observed morbidity was 18.4 percent and the mortality 1.5 percent. It is very difficult to obtain a reasonably accurate determination of the number of sick sheep in a flock since the observations are usually made during the onset rather than after the termination of the disease outbreak. It is apparent, therefore, that the morbidity and mortality estimates are too low.

Since a routine serological test has not yet been perfected, the laboratory diagnosis of bluetongue in this country is based on recovery of the causative virus from blood of infected sheep. This requires the use of susceptible test sheep. A white-faced, nonpigmented-hoofed sheep is highly desirable for this purpose. Susceptibility to bluetongue of a source flock is demonstrated by testing a representative number of animals prior to making further purchases.

In the routine procedure for diagnosing bluetongue, test sheep are moved to an isolation room along with two sheep that are to serve as the in-contact temperature controls. Blood samples are collected, and preinoculation temperatures taken of all animals. Test sheep are inoculated either subcutaneously or intravenously with four ml. of the unknown blood sample. Temperatures are taken, and observations made twice daily for 14 days.

A positive reaction is, as a rule, characterized by the appearance of a febrile response of 150° to 108°F., which quite often occurs from the sixth to eighth day postinoculation. This is usually accompanied by some or all of the following symptoms: a nasal discharge, salivation, edema of the lips and hyperemia or cyanosis of the nasal and buccal mucosa and occasionally of the tongue. Excoriation of the epithelium at the commissures of the lips and ulceration of the buccal mucosa frequently occur. Other manifestations commonly observed are inappetence, lassitude, respiratory difficulties, and loss.

* An anticoagulant preservative solution of potassium oxalate five gm., phenol five gm., glycerin 500 ml., and distilled water 500 ml.
of body weight. The two sheep used for temperature controls should remain normal throughout the course of the test.

Blood for future studies is routinely obtained from the inoculated sheep on the seventh day postinoculation and at or near the peak of the thermal reaction. This blood is used as the inoculum for second serial passage tests in instances where the sheep injected with the field sample failed to show an apparent reaction. The procedure followed for the subpassage test is the same, except for the inoculum, as the method described above for the original test.

Using these criteria, a diagnosis of bluetongue has been made in 48 of 100 flocks tested at the Denver station. The validity of the test was confirmed by the fact that sheep representing 37 of the 48 flocks did not manifest any reaction when challenged with known bluetongue virus 21 days or more after the initial injection of the field sample. Sheep used as virus controls gave uniformly good indications of a typical reaction, and in-contact temperature controls remained normal. In subsequent tests the animals used for virus controls were immune to challenge, while those used for temperature controls reacted to challenge.

The 48 bluetongue virus isolations which have been made were from flocks located in the following States: Arizona seven, California five, Colorado seven, Kansas two, Missouri three, Nebraska one, Nevada one, New Mexico six, Oklahoma two, Oregon two, Texas two, and Utah 10. Because of the wide geographical distribution of the disease, the possibility of different strains of virus being involved must be given consideration. One hundred and four test sheep that had recovered from clinical bluetongue showed no reaction when cross-challenged with known bluetongue virus. A total of 12 viruses, three from the University of California, were used which had been proved by reciprocal cross-protection tests to confer the same basic immunity (10). No evidence that more than one strain of bluetongue virus exists in this country was demonstrated in this study.

The symptoms and lesions of bluetongue that have been noted in experimental animals held under confined and protected conditions have been for the most part less severe than those that have been observed in field cases. Moreover, from our field observations and evaluation of case histories it has become apparent that lesions and death losses are likely to be less severe in farm flocks that are well fed and cared for than flocks maintained on drought-stricken or desert ranges. As a result of observations we have come to the conclusions that stress is an important predisposing factor in determining the severity of the disease.

That bluetongue is stable was attested to by the South Africa report (11) that bluetongue virus vaccine (live virus in sheep blood stored in OCG solution—Theiler's vaccine) which had been inadvertently lost in an office for 25 years was proved infective when inoculated into sheep. During an outbreak of clinical bluetongue in field sheep, we collected blood in OCG solution and stored it at 5° C. for 34 months before inoculating a test sheep subcutaneously with four ml. of the material. Temperatures of 106.4° and
106.7°F. on the ninth and tenth days, respectively were the first indication of a reaction. This was followed by more severe symptoms and lesions than we have commonly observed in reactor sheep confined in the isolation rooms. The virus recovered was proved on challenge to be bluetongue virus.

The flock histories, which were obtained from the owners of the 100 suspected bluetongue-infected flocks of sheep and submitted to us with the blood samples, were studied to determine their significance to the findings obtained by testing samples. The results are shown in Table I. The correlation be-

### TABLE I

Correlation Between Flock Histories and Results of Test

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between the case history and the findings upon testing the blood samples points to the importance of getting a complete history on the suspected bluetongue-infected flock before selecting the animals from which to collect blood samples. The history submitted in some cases definitely indicated the presence of clinical bluetongue in the flock. However, the samples submitted for test were collected from animals that had probably passed through the acute febrile stage of the disease and had apparently produced sufficient antibodies to neutralize the virus. It appears that this fact was substantiated by the failure to isolate the virus from some of these animals, even though second and third subpassage tests were conducted with sheep which subsequently reacted to challenge with a known bluetongue virus. It would save much time, work, and expenditure of test animals if the blood samples to be submitted for virus isolations were collected from sheep during the acute febrile stage of the disease, instead of after the typical symptoms and lesions are apparent to the naked eye.

Blood or serum samples from cattle in Delaware, Maryland, Kansas, Oklahoma, Texas, Colorado, New Mexico, Arizona, and California were also received. Some of the cattle in the southern and western states were confined to pastures with sheep from which bluetongue virus was isolated, to pastures separated by a fence from infected sheep, or to ranges or pastures where known bluetongue had previously occurred in the area. Sheep were inoculated with the field bovine blood samples and subpassages were made, but bluetongue virus was not recovered. The sheep used in the tests responded later to inoculation of the known virus. To date, we have not isolated bluetongue virus from cattle.
REFERENCES

1. MILLER, DONALD: Personal communication to C. L. Davis, Oct. 29, 1954.


THE FIELD USE OF MODIFIED LIVE VIRUS VACCINE

C. L. CAMPBELL, D.V.M.*
Tallahassee, Florida

Lest any of you should get the impression from the title of this paper that I am professing to be an expert in the use of modified live virus vaccines, I hasten to state that this report merely involves experiences with these products in the State of Florida from which you may make your own evaluations. I fully realize that Florida cannot be classified as a leading swine producer in comparison with the Corn Belt States; however, because of some of the unique aspects involved in the use of anti-hog cholera products in Florida, valuable data on the disease can be gathered which would be difficult, if not impossible, to obtain in many of the mid-western states. You may recall that this fact was given paramount consideration by your Committee on the Nationwide Eradication of Hog Cholera in establishing a pilot test project in Florida.

In order that you may have a better understanding of our hog cholera program I would like to give you a little background. First, all cholera vaccination conducted in the state is under the direct control of the Florida Livestock Board, anti-hog cholera products being furnished to swine producers through supervised administrators without cost. The program of supplying free hog cholera serum and virus to bonafide Florida farmers was begun over 30 years ago when our State Legislature appropriated some $25,000 for the purchase of these products to combat high cholera losses. Through the use of these products the incidence of hog cholera was materially reduced and a stimulus was thus created for the production of better type swine. As a result the Florida hog population has increased to somewhat over a million and the breeding in these animals has progressed from the “piney-woods” open range hog to the present day type exhibiting the quality and conformation existent in corn belt swine. Throughout these years the legislature continued its appropriations for cost-free products up to a quarter of a million dollar level in 1956.

Inasmuch as the Florida Livestock Board is accountable for every cubic centimeter of serum, virus, or vaccine purchased, rigid restrictions are placed upon each veterinarian or county agent who is authorized by the Board to administer the products to submit detailed records on his work. These involve reporting name, address, and number of swine vaccinated for each owner, as well as the amount of serum, virus, or vaccine administered; reporting of cholera cases and witnessed declaration of the destruction of expired virus or reconstituted vaccine not used during the day. Routinely, our program director supervises the administrators in the proper handling and

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refrigeration of products in the office and field, in addition to the maintenance of aseptic techniques on the farm.

With these controls in effect we are able to maintain rather accurate records and to make fair evaluations of the progress of our program. In addition we are experiencing very few "breaks," as a result of which the incidence of cholera is quite low in vaccinated herds. Actually, up to June 30, 1957, we have had but one "break" reported in a herd vaccinated with modified live virus since we started using the product in the latter part of 1954. It is my opinion that these results are attributable not only to the rigid controls which I just mentioned, but also to two other factors. First, when the dried vaccine is reconstituted preparatory to vaccination the entire contents of the vial are used then and not carried over for subsequent use, and second, a minimum of 15 cc's. of serum is used with each dose of vaccine regardless of whether it is of porcine, rabbit, or tissue origin. Of course, the amount of serum is increased if the animals are being sold through a market or if there is other history of exposure.

During the three-year period between July 1, 1954, the date when the Livestock Board first began distributing vaccine, and June 30, 1957, 1,056,947 swine were vaccinated in Florida—748,755, or 70.8 percent of which received modified live virus. The acceptance of these recently developed products increased markedly in this short span of months as evidenced by the fact that during the first six months of the period 12 percent of the total vaccinations were with modified virus, while during the last six months 82 percent of the total vaccinations were with modifieds. The remaining 18 percent in this latter period represent almost in toto county agent vaccination in which virulent virus and serum was used. As a matter of information, our Board adopted a policy when we first began purchasing modified virus to restrict it to graduate veterinarians, since it was felt that there was still much to be learned concerning its usage. Consequently, county agent vaccination has been limited until recently to serum and virulent virus. Last month, however, by virtue of discontinuing purchase of the product and by prohibiting any commercial sales within the State, Florida has now "outlawed" virulent virus. Although this action might have been taken at any previous time, the Board felt that further experience should be gained with modified viruses before taking this step. As a result of this action county agents are now administering inactivated hog cholera vaccine.

Earlier, reference was made to the establishment of a hog cholera pilot test project in Suwannee County, Florida. This program was brought into full realization with completion of laboratory facilities in Live Oak by the Federal Government at the turn of this year. The purpose of the project, which will be of vital benefit to swine growers throughout the country, is to determine what disease eradication methods may be considered effective and practical when applied on a national scale. The Florida station is presently centering its attention on swine which have previously been vaccinated with modified viruses.
Field reports which were compiled from the pilot test area during 1956 have revealed some interesting preliminary data. Forty thousand seven hundred and twenty-seven swine were vaccinated in Suwannee County during the year, all with modified live virus vaccine, representing 11.3 percent of the total swine vaccinated in Florida in 1956. Of the 439 hog cholera deaths reported in the State during this time, none occurred in this supervised county. In contrast to this, during the year preceding the inauguration of the pilot test area, 25 percent of the State’s cholera was reported from Suwannee County. The scientific significance which may be drawn from this information would probably be more in the field of conjecture because of the many variables and unknowns involved. However, as a regulatory official, rather than as a technician, I can recognize its significance to the average farmer in the area who is quite enthusiastic concerning these results. One can more fully appreciate this enthusiasm from the fact that during the period from July of 1955 through June of 1957, during which period modified virus was used exclusively in the county, all three practicing veterinarians there reported that there was not even a case of slight postvaccinal reaction.

The success attained to date in the pilot test county is due in large part to the efforts and cooperation extended by the Suwannee County practitioners. Not only have they worked fully with the Livestock Board and United States Department of Agriculture in promptly filing reports on their vaccination activities, but they have also taken on extra work without compensation except in the satisfaction of a job well done in submitting case history data, including temperature of at least 10 percent of the herd, in each herd vaccinated. They have even identified each animal so that in later months when challenged for established immunity it could be correlated with the given serial of vaccine which was used on it. This is no simple task in the life of a busy practitioner considering the approximate annual vaccination of some 40,000 swine in over 1,200 herds.

Although I do not wish to report in detail on research data which is being developed at the Hog Cholera Research Station at Live Oak, I do want to advise you of the progress which is being made there. This facility encompasses some ten acres of land upon which are located a well-equipped laboratory and office, a challenge barn, an immune pig barn, a susceptible pig barn, and 12 experimental pens. The staff is under the able direction of Dr. M. R. Zinober who, for a number of years, has devoted himself to hog cholera research. I hope that by the time of the next meeting of this organization Doctor Zinober will have accumulated sufficient data to be able to present to you some conclusive results on his work which may be used as at least a partial pattern in launching an all-out eradication program. It should be realized that the following figures, however, merely represent the work of approximately five and one-half months and because of the relatively small number of swine involved cannot be used as criteria except in the indication of a possible trend. In the establishment of the pilot test area a Memorandum of Understanding was formulated which in part provided that “a large testing program will be conducted to determine the immunizing value of the different
types of vaccines,” and further that “two or more percent of the vaccinated swine from all vaccinated herds (in the county), the total number of which shall not be less than two from any one herd, shall be acquired for virulent virus challenge.” Actually, 5.1 percent of the vaccinates are being challenged which, of course, enhances the statistical value of the results. All three types of modified virus are being used in Suwannee County in relatively equal proportions, and it is interesting to note the remarkably similar results in immunity response to challenge. In an immunity development system formulated by Doctors Mott, Torrey, and Zinober, field vaccinated swine challenged to date in the research station have exhibited a very high degree of immunity. In tests involving swine vaccinated with porcine origin, rabbit origin, and tissue culture vaccines 620 animals from 335 herds have been challenged through November 6. Of this number, 595 hogs from 309 herds were shown to be adequately protected against injections of known virulent virus. This may be stated in other terms as adequate protection in 96 percent of the swine in 92.2 percent of the herds. Although a few of the animals in the tests had been vaccinated for as long as two years or more prior to challenge, the average number of days post-vaccination was 255.3.

Since I do not profess to be a statistician I cannot even venture a guess as to the relative merits of a project evaluation based upon the foregoing figures. It is not, however, difficult for me to evaluate a program which allows the farmer to enjoy greater profits through the marketing of increased numbers of healthier hogs. This, of course, is the ultimate objective in the eradication of hog cholera. The results which we have realized to date in a program incorporating the use of modified live virus vaccines indicates that we have taken a firm step in reaching our objective.

REFERENCES


DISCUSSION

PRESIDENT GOOD: Thank you, Doctor Milligan, for your report.
Does anyone have a question to ask either Doctor Campbell or Doctor Milligan?

DOCTOR KUTTLER: I would like to ask Doctor Campbell if he anticipates a compulsory program in the States, in view of the very satisfactory results he has had in Suwannee County.

DOCTOR CAMPBELL: Do you mean a Statewide compulsory program? No, we haven’t anticipated that so far because of the relatively little work that has been done in Suwannee County. As I stated, although the buildings were completed the beginning of the year, because of flood conditions and some
difficulties in obtaining the personnel and getting them down there, they have been in operation for only five and a half months, and to date the results are not really conclusive.

Doctor Kuttler: I was also wondering about the relationship between the county agricultural agent and the veterinarian. You mentioned county agents. Have they been administering this vaccine?

Doctor Campbell: Not in the pilot test counties, but in counties not provided with practicing veterinarians.

Voice: In your State program does the veterinarian charge the farmer for administration of the vaccine, or is he paid by the State?

Doctor Campbell: The veterinarian charges the farmer for his services, so much per head—usually 20 to 30 cents.

Voice: The usual veterinarian-client relationship has not been interrupted?

Doctor Campbell: That's right.

Voice: Doctor Campbell, you mentioned that at least 10 percent of each herd had temperatures taken on them. By that method do you determine that a large number of the herds are unsuitable for vaccination?

Doctor Campbell: There have not been a great number of herds which we did not consider good vaccination risks.

Voice: You vaccinated, regardless of the temperatures found?

Doctor Campbell: No. If the temperature is up, the veterinarian, of course, advises the client to hold off and determine what might be causing the temperature elevation. He usually arranges for vaccination at a later date.

Doctor Kuttler: I believe you said the State furnishes the vaccine?

Doctor Campbell: That is true.

Voice: In that regard, then, the veterinarian does not have a choice of the type of vaccine he is going to use, but only what the State provides; is that right?

Doctor Campbell: The veterinarian has the choice of the three types, whether it be porcine, rabbit or tissue origin, but not a given brand. We purchase these products on a low bid basis, of course.

Voice: Do you think that is necessary for a control program, rather than prescribing that the veterinarian use not virulent virus but some modified virus, and let him purchase it on the open market? Do you suggest that as a necessary thing for a control of the program?

Doctor Campbell: Do you mean the fact that it be given without cost to the owner?

Voice: Yes.

Doctor Campbell: Well, it certainly has enhanced the number of vaccinations we have had.

Doctor Mott: Does the farmer have any choice in the vaccine that is used in his herd?

Doctor Campbell: Usually he doesn't care. He says, "It's up to you, Doctor. Use whatever you want to use."

We attempt to equalize the amount of vaccine that is used in the county to about 33 1/3 percent of each particular type of product being used.
VOICE: This question may have been answered, but I would like to ask it again. Is the use of serum ever indicated in conjunction with the modified live virus?

DOCTOR CAMPBELL: The use of serum with modified live virus? We use it with all modified live virus.

DOCTOR KUTTLER: I have another question. Is it your thought that the present program will succeed in a sufficient percentage of owners placing their hogs under supervision to complete eradication?

DOCTOR CAMPBELL: The trends certainly indicate that in that county. We are very well pleased with the results. The owners have not found any inconvenience in being under the program in that county as opposed to the time when they were not under it.

DOCTOR MOTT: Regarding the use of hog cholera antiserum with these modified live viruses, I wonder if you would say a word about the minimum and maximum dosage recommended, and the reason for both the minimum and maximum dosage.

DOCTOR CAMPBELL: We have felt that there is nothing cheaper than serum in Florida, it being free. There is no reason not to use serum with any of the products. We do know that in many cases we do not have a complete history concerning previous exposure, and therefore it is foolish to take a chance and not use serum with the product.

Our minimum dose is 15 cc. That is what we first recommended when we began to purchase the vaccine back in 1954, with the advice to the veterinarians that they graduate the dosage upward depending upon the exposure.

We feel that any animal going through a market certainly constitutes exposure, the result being that the amount of serum is elevated above the 15 cc. level; usually it is doubled or better.

DOCTOR MOTT: We also suggested keeping the dosage down, not to exceed 15 cc. on account of getting information on it.

DOCTOR CAMPBELL: That is true. We could have given more. In fact, the veterinarians in the pilot test area were averaging closer to 20 cc. in the first year or year and a half. Therefore, in order to get a better determination of the relative immunizing properties of lesser quantities of serum, we have instructed them to cut down on the use of the serum to a 15 cc. level, and our results are proving to be very satisfactory with the minimum amount.

DOCTOR ROSNER: Are there any animals in a herd in this Suwannee County project left unvaccinated, or is each and every animal in the herd vaccinated?

DOCTOR CAMPBELL: Usually they are all vaccinated. However, there are times when you can’t get all of your hogs up, as you well know. For the most part, however, the entire herd is vaccinated.

VOICE: Does the age and weight of the hog act as a determining factor in the amount of serum that you use with the modified live virus?

DOCTOR CAMPBELL: Not usually. Usually the previous exposure is the governing factor.

PRESIDENT GOOD: Are there any other questions? If not, thank you very much, Doctor Campbell.
REPORT OF THE COMMITTEE ON NATIONWIDE ERADICATION OF HOG CHOLERA


The Committee wishes to again call to your attention its recommendations made before this Association last year and as published in the proceedings of 1956. We wish to reaffirm those recommendations and urge that steps be taken by this Association and the individual states to put into effect the provisions of these recommendations so that an effective Hog Cholera eradication program can be established at the earliest possible date. We also wish to reaffirm the reports made by this Committee in the past.

Progress has been made during the past year toward carrying out some of the Associations recommendations as evidenced by the fact that the number of states that have outlawed the use of virulent hog cholera virus, either by laws or regulations, have increased from eight as of a year ago to a total of 16 at the present time.

A bill has been introduced in the Congress of the United States, that if enacted, would control the production and distribution of Virulent Hog Cholera Virus at a national level. This bill is now being held in the House Agricultural Committee. We urge this Association to lend its every effort toward a favorable report of the Agricultural Committee on this bill. We feel that the holding of this bill in Committee has caused some States to lax their efforts toward the passage of State legislation directed at the control of fully Virulent Hog Cholera Virus.

We urge that the individual States take such action as is necessary to call to the attention of their representatives in Congress the importance of the passage of the bill now held in Committee. We also urge that the individual States renew their efforts toward the passage of such legislation at a local level.

Your Committee again wishes to call your attention to the publishing of a pamphlet on “What is Known About Hog Cholera.” Information to be contained in this pamphlet has been revised and brought up-to-date and is now ready for publication. We urge that this publication be printed at the earliest possible date.

The use of fully Virulent Hog Cholera Virus has decreased each year since the beginning of the production of Attenuated Vaccines so that at the present
time less than 20 percent of the swine that are being immunized are being immunized with fully Virulent Virus and Serum. The Committee feels that further reductions can rapidly be made in this percentage if the administration of an adequate dose of antiserum is required with all of the present modified live virus products.

The United Kingdom has expressed its willingness to purchase pork products from those states in which Vesicular Exanthema does not exist and which have taken the necessary steps to ban the use of Virulent Hog Cholera Virus. This opens a world market to those states and is of importance to their agricultural economy. We urge all states that are in position to do so to take advantage of this opportunity.
A survey of helminth infection in Wisconsin market-weight swine (1) based upon examinations made during 1955 and 1956 established that animals acceptable for human consumption carried an average of 463.3 worms of 3.5 genera to the packing plant in Madison.

A survey (2) on the prevalence of swine Ascaris conducted in 1920 reported 41 percent of swine intestines collected from slaughter houses in Chicago carried this worm. A 1934 survey (3) in southeastern swine established a 74 percent prevalence of swine Ascaris. A 1945 survey (4) of Georgia swine established a prevalence of 68 percent for swine Ascaris. Based on a fecal smear technic (5), it was reported in 1949 that 35 percent of 180- to 225-pound hogs packed in Chicago were infected with Ascaris. The Wisconsin survey (1) demonstrated the incidence of Ascaris in the population examined to be 65.4 percent. These surveys over a 36-year period do not indicate a decline in Ascaris population.

Because of the number of animals involved in the surveys of swine Ascaris prevalence the data reviewed above are not considered to record a rise or fall of Ascaris population in swine. The fact that the Ascaris population remains in American swine and has significance in connection with anthelmintics will be considered.

Continued literature citation perhaps is unnecessary in order to establish the ability of the swine Ascaris to injure its host. Few data are available which have been collected directly from tests designed to measure the ability of the swine Ascaris to interfere with production, i.e., feed conversion efficiency.

Certain other species of swine helminths have had their prevalence recorded (3, 6, 7). It can be noted, however, that the 1957 survey (1) established the presence of the great majority of the different species of swine helminths in a northern state, Wisconsin, in mixed, not pure, infections. The concept of effective geographical barrier to helminth prevalence so widely held in previous years must now be discarded in the light of the development of the modern transportation methods of livestock.

It is unfortunate that attempts were made in the past to assign arbitrary standards of “importance” to the various swine helminths. Such “standards” resulted in the use of anthelmintics to treat specific worm infections. The fact of the matter is, supported by all past helminth surveys, that helminth infection in livestock is mixed and not one, pure, “important” species.

* Department of Veterinary Science, University of Wisconsin, Madison.
One great difficulty in veterinary parasitology in the past has been insistence that specific, severe, "important" parasitism could be diagnosed clinically. This was accompanied by insistence that there must be devised still more clinical methods which could diagnose the separate species of helminths within a given swine. There exists a school of parasitologists today which maintains that the "important" parasitisms, from the standpoint of livestock production, are subclinical in nature and not susceptible to clinical diagnosis.

The earlier anthelmintics for swine, which succeeded each other in turn as the drugs of choice for the treatment of Ascaris infection almost exclusively, could be used only in full curative amounts. These anthelmintics were oil of chenopodium, sodium fluoride, the two cadmiums and the piperazines. They largely have been used to remedy the physical distress of animals diagnosed to be severely affected by Ascaris. It was possible, although the attempt was not made widely, to use these same anthelmintics in a preventive attack against the swine Ascaris even though they had to be used in full curative amount. Preventive treatment with these same anthelmintics could have been attained if the treatments had been timed with relation to the life cycle of the swine Ascaris instead of to the physical distress of the infected animals.

That periodic, full curative treatment for swine Ascaris had not succeeded in the control of the infection, in the face of increased swine population, is obvious in the comparison of swine Ascaris population recorded in the surveys of 1920 and 1957.

When helminth infections came to be considered from the standpoint of livestock production it was possible to devise continuous preventive systems of subtherapeutic anthelmintic administration. These systems largely have been developed within the past decade. Because these systems have been accompanied by development of broad-spectrum anthelmintics a consistent action against the helminth infections in the swine which constitute the source of pork production now can be established.

It is probable that by mid-1958 over half of the swine destined for market production in that year will have received preventive anthelmintic therapy in either complete feeds or supplements as a regular device on the part of management to increase the efficiency of swine production. The new broad-spectrum anthelmintic, Hygromycin-B (8), will be the drug of choice.

With continuous preventive anthelmintic treatment the effectiveness of the treatment of swine with subclinical infections will be measured by the criteria of efficient livestock production and not by the criteria of "worms killed." This is not to say that continuous preventive treatment with the new broad-spectrum swine anthelmintic does not remove worms from swine. The statement means that the swine parasite problem will not be reduced until it has been established that it is profitable for a swine farmer to do so, with his best pigs and not with the pigs destined to die on the farm.

Work in progress with Hygromycin-B in parasitology laboratories indicates that it is now possible to eliminate somewhat more than 80 percent of the
normal helminth population in market swine. The implications of this development carry over among the virus parasites which constitute such an important part of the swine problem.

The swine lungworm was identified as a reservoir and intermediate host for swine influenza virus in 1941 (9). Relationships between Ascaris and vesicular stomatitis (10) apparently are being established. It is known that other relationships between Ascaris and swine respiratory infections, and Ascaris and swine cholera are being studied. Such research identifies a derived pathology of helminths.

It is pertinent to this discussion to observe that prevention of virus infection in swine will be linked to prevention of helminth infection.

REFERENCES
FURTHER STUDIES ON THE RESIN AGGLUTINATION TEST FOR VESICULAR STOMATITIS AND HOG CHOLERA

D. Segre, D.V.M., M.S., Ph.D.

University of Wisconsin,
Madison, Wisconsin

A new serologic procedure for detecting viral antigens and their antibodies was described in a previous paper (1). This procedure, which was named resin agglutination (RA) test, is based on the ability of viral antigens to cause clumping of particles of an anion exchange resin coated with the specific viral antibodies. Titration of viral antibodies was accomplished by means of a resin agglutination-inhibition (RAI) test, in which the ability of a test serum to inhibit agglutination by a known antigen preparation was taken as a measure of the level of specific antibody in the test serum.

The RA and the RAI tests were successfully applied to two viruses of swine, hog cholera and vesicular stomatitis (1). Additional work with vesicular stomatitis (2) indicated a correlation between the RAI and the serum neutralization (SN) tests.

Paper NS 236 from the Department of Veterinary Science, University of Wisconsin, Madison, published with the approval of the director of the Wisconsin Agricultural Experiment Station.

These investigations were supported in part by grants from the United States Department of Agriculture, Agricultural Research Service, and from the National Institutes of Health, United States Public Health Service.

Further observations were made on the application of the RA and the RAI tests to the diagnosis and epidemiology of vesicular stomatitis and hog cholera. These observations constitute the basis for this report.

MATERIALS AND METHODS

The materials and methods used for the RA and RAI tests were described in previous publications (1, 2). However, the procedure of the RA and RAI reactions will be summarized here.

A small amount of the anion exchanger, Amberlite IRA-400, was finely ground in a mortar, suspended with distilled water in a glass cylinder, and allowed to settle for 30 minutes. The supernatant fluid was then transferred to a tube and centrifuged for five minutes at 1,500 r.p.m. The supernate was discarded, and the sediment resuspended in a small volume of 1N NaOH. This suspension was again centrifuged and the sediment resuspended in distilled water. The excess NaOH was eliminated by repeated washings with distilled water. The final sediment was resuspended in 0.1 molar tris (hydroxymethyl)aminomethane (tris) and the density adjusted to five percent light transmittance at a wave length of 450 millimicrons.
The specific antibody (gamma globulin from an animal hyperimmunized to hog cholera or vesicular stomatitis) was then diluted 1:1000 with 0.1 molar tris. Equal volumes of solution of gamma globulin and of suspension of resin particles were mixed in a tube and shaken during a period of 10 minutes. After centrifugation, the antibody coated resin particles were re-suspended in 0.1 M tris.

In the RA test, the virus-containing material was diluted serially with 0.1 M tris containing two percent bovine serum. One drop of each dilution was placed on a glass plate. One drop of sensitized resin particle suspension was added to each drop of virus-containing material. After mixing and rotation of the plate, the test was read. The titer was expressed as the highest dilution of virus-containing material that caused clumping of the resin particles.

In the RAI test, a virus concentration was employed which was four times greater than its RA titer. A volume of the virus preparation was added to equal volumes of serial dilutions of the test serum. A drop from each of these mixtures was then mixed on a glass plate with a drop of the sensitized resin particle suspension. After mixing and rotating the test was read. The antibody titer of a test serum was expressed as the highest serum dilution that completely inhibited agglutination.

RESULTS

In the course of studies on hog cholera and vesicular stomatitis some general observations were made on the procedures employed in the RA test. These observations will be reported first. The results obtained with various materials tested for hog cholera and vesicular stomatitis will follow.

Preparation of distilled water. It was found that the purity of the distilled water was essential. Even small quantities of "foreign" ions in the distilled water caused non-specific agglutination during the repeated washings of the resin. Water for use in the RA test is now treated as follows: the water is first passed through a commercial de-ionizer, then through a column of Amberlite IRA-400 (the same anion exchanger that is used in the test), and finally it is twice distilled in all glass stills.

Non-specific agglutination. When whole, defibrinated swine blood was tested for hog cholera virus, it was noted that both virus-containing and normal blood caused clumping of the sensitized resin particles. It was found that the non-specific agglutination was due to the ghosts of the red blood cells present in normal blood. Thorough centrifugation or filtration through an asbestos pad was necessary to remove the ghosts of red blood cells and the non-specific agglutination.

Citrated blood was also found to cause non-specific agglutination. Citrated blood clotted when mixed with the diluent used in the test, thus inducing clumping of the resin particles.
RA titers obtained using different lots of Amberlite IRA-400. A preparation of hog cholera virus was titrated using different lots of Amberlite IRA-400. The titers obtained varied over a considerable range. (Table 1.)

**TABLE 1**

*Comparative Titration of a Hog Cholera Virus Preparation Using Six Lots of Amberlite IRA-400*

<table>
<thead>
<tr>
<th>Lot Number</th>
<th>RA Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>760272</td>
<td>40*</td>
</tr>
<tr>
<td>11-9187</td>
<td>80</td>
</tr>
<tr>
<td>7557</td>
<td>320</td>
</tr>
<tr>
<td>8011</td>
<td>640</td>
</tr>
<tr>
<td>11-9739</td>
<td>1,280</td>
</tr>
<tr>
<td>9772</td>
<td>1,280</td>
</tr>
</tbody>
</table>

* Reciprocal of the dilution of the test material prior to mixing with the sensitized resin particles.

**Effect of storage on the sensitized resin particle suspension.** A preparation of hog cholera virus was titrated immediately after preparation of the sensitized resin particles and after storage of the latter overnight at five C and at -20 C. When the sensitized resin was stored overnight at five C, there was a decrease in titer from 1:128 to 1:8. The suspension of sensitized resin particles that was stored in the frozen state at -20 C failed to agglutinate when tested after thawing. It was concluded that the sensitized resin particles had to be used on the same day of their preparation.

**Correlation between SN and RAI titers using serums from animals immune to vesicular stomatitis.** Forty-three serum samples with various levels of antibodies neutralizing vesicular stomatitis virus were tested with the RAI test. Twenty-nine serum samples were of bovine origin, nine from swine and five from raccoons.

**The RA test as a diagnostic method for hog cholera.** A limited number of specimens were received from the Animal Disease Diagnostic Laboratory of the State of Wisconsin, Madison, for diagnosis of hog cholera. In most cases, the materials submitted came from herds of swine where a diagnosis of hog cholera could not be made on the basis of clinical and necropsy findings. Materials from typical outbreaks of hog cholera were not generally submitted.

Brief case reports, the diagnoses of the attending veterinarians and the results of the RA tests are shown in Table 2.

1 Drs. A. F. Krohn, B. Mendlowski and E. D. Baker kindly supplied these materials, the case histories and diagnoses.
### TABLE 2

**Case Reports, Diagnoses of the Attending Veterinarians and Results of the RA Tests of 12 Outbreaks of Swine Diseases in Wisconsin**

<table>
<thead>
<tr>
<th>Case History</th>
<th>Diagnosis</th>
<th>RA Test for Hog Cholera</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Part of herd vaccinated previously. 5-6 died. The rest apparently recovered.</td>
<td>Swine influenza and pneumonia</td>
<td>Positive</td>
</tr>
<tr>
<td>2. Twenty-five out of 83 pigs survived. Treated and vaccinated after diagnosis was made.</td>
<td>Hog cholera</td>
<td>Positive</td>
</tr>
<tr>
<td>3. A typical hog cholera, encephalitic form. Six out of 34 pigs survived. Treated and vaccinated after diagnosis was made.</td>
<td>Hog cholera</td>
<td>Positive</td>
</tr>
<tr>
<td>4. All well and some sick pigs vaccinated with modified virus and serum early in the outbreak before specimens were obtained. Fifty-one pigs died out of about 185.</td>
<td>Hog cholera</td>
<td>Negative</td>
</tr>
<tr>
<td>5. Septicemic lesions. Herd vaccinated and treated for erysipelas after diagnosis was made. One month later the herd was still well.</td>
<td>Erysipelas</td>
<td>Negative</td>
</tr>
<tr>
<td>6. About 70 pigs involved in an outbreak of erysipelas. Pigs treated and recovered then became sick again. Retreated for erysipelas, with no response.</td>
<td>Hog cholera</td>
<td>Positive</td>
</tr>
<tr>
<td>7. Four pigs died with massive hemorrhages in digestive tract and subcutaneously. No more pigs affected.</td>
<td>Suspected moldy feed</td>
<td>Negative</td>
</tr>
<tr>
<td>8. Respiratory illness with elevated temperature over a period of several days. WBC normal, RBC low. Lesions suggestive of hog cholera. Later information was that most of the pigs died after prolonged illness regardless of treatment.</td>
<td>Suspected eperythrozoonosis</td>
<td>Positive</td>
</tr>
<tr>
<td>9. Six sows died after acute illness with very high temperature. Hemorrhagic lesions observed at necropsy. Numerous baby pigs also died. One hundred and thirty feeder pigs which had been vaccinated for hog cholera remained well. Pasteurella isolated from lungs of sows.</td>
<td>Pasteurellosis</td>
<td>Positive</td>
</tr>
<tr>
<td>10. Respiratory and nervous disturbances. Streptocci found in brain cultures. Cellular infiltration and perivascular cuffing seen in section of brain.</td>
<td>Virus pneumonia and encephalitis</td>
<td>Negative</td>
</tr>
<tr>
<td>11. Four pigs in a herd of 150 died, a few more showed signs of illness.</td>
<td>Atrophic rhinitis</td>
<td>Negative</td>
</tr>
<tr>
<td>12. Ten feeder hogs became sick with signs and lesions of hog cholera.</td>
<td>Hog cholera</td>
<td>Positive</td>
</tr>
</tbody>
</table>
In eight out of 12 cases there was agreement between the diagnosis of the attending veterinarians and the result of the RA test. In four of the eight cases a diagnosis of hog cholera was made, while in the other four cases the disease was not hog cholera.

In one case (No. 4) with diagnosis of hog cholera, the RA test was negative. In three cases (No. 1, 8 and 9) with positive RA test, a diagnosis other than hog cholera was made.

DISCUSSION

Purity of the distilled water was found to be very critical for the RA test. This is not surprising in view of the fact that the anion exchanger, Amberlite IRA-400, has greater affinity for a variety of anions than for the carboxyl ions of gamma globulin. Anions present in the distilled water would bind the reactive groups on the resin thus preventing adsorption of gamma globulin. By passing the water through a column of Amberlite IRA-400 all anions which would combine with this resin were presumably removed. The procedure for obtaining distilled water that was outlined in the section on results was satisfactory in our experience.

Stroma of red blood cells caused non-specific agglutination of the resin particles. Binding of red cell stroma by an anion exchange resin was described by Isliker (3). Non-specific agglutination can probably be explained on the basis of linkage between resin particles through red cell ghosts.

A wide variation in the RA titers of a virus-containing preparation was noted when different lots of Amberlite IRA-400 were used. These results can probably be explained on the basis of variations in the binding capacity of different lots of resin. The lots with lower binding capacity would not adsorb as much gamma globulin as other lots, and a lower RA titer would result. As a practical consequence, a number of batches of Amberlite IRA-400 should be tried and the ones giving highest RA titers selected.

Storage of the antibody-coated resin particles was not feasible. This would have been very desirable because it would have permitted the preparation of diagnostic reagents to be supplied to laboratories and practitioners. Work is in progress to develop such diagnostic reagents. Two possibilities are under consideration. One is that the linkage of gamma globulin to the resin was too weak and dissociation occurred rapidly. The other is that the high pH used in the RA test caused a slow denaturation of gamma globulin.

Comparative titrations with the RAI and SN tests were made on 43 serum samples that neutralized vesicular stomatitis virus. Only three serum samples, or seven percent were positive with the SN and negative with the RAI test. This is considered good agreement between the results obtained with the two procedures. The correlation was especially good with nine swine and five raccoon serums. Testing of a larger number of serum samples should give more valid results. It is felt, however, that the results obtained so far warrant the use of the RAI test as a screening procedure for epizootiological surveys of vesicular stomatitis.
Experience with the RA test as a diagnostic procedure for hog cholera was in general satisfactory. In cases Nos. 2, 3, 6, and 12 a diagnosis of hog cholera was made by the attending veterinarians and was confirmed by the positive results of the RA test. A diagnosis other than hog cholera was made in cases Nos. 5, 7, 10, and 11 with a negative RA test. In the other four cases there was disagreement between the clinical diagnosis and the results of the RA test. Case No. 1 was diagnosed as swine influenza. The fact that some of the pigs recovered makes it unlikely that the disease was hog cholera. The positive RA test might have been due to vaccinal virus present in the samples tested, since part of the herd had been recently vaccinated. Case No. 4 was diagnosed as hog cholera, but the RA test was negative. However, the pigs in this herd received hog cholera antiserum before specimens were obtained for serology. Hog cholera antibodies were therefore present in the specimens and very probably made the virus undetectable with the RA test. A similar situation was encountered in case No. 12. Specimens from two pigs were submitted, one of which had received hog cholera antiserum. The RA test was negative with material from this pig, but was positive with material from another pig which had not been given hog cholera antiserum. Cases Nos. 8 and 9 probably represented outbreaks of hog cholera, although a different diagnosis was made by the attending veterinarians. In both cases the RA test was positive. In case No. 8 most of the pigs eventually died, regardless of the treatment used. In case No. 9, 130 pigs which had been vaccinated for hog cholera remained healthy, whereas the unvaccinated sows and baby pigs died. Pasturella organisms were isolated from the lungs of the sows, but these organisms are known to be often associated with hog cholera.

It appears that the real disagreement between RA test and clinical diagnosis should be limited to case No. 1. It is felt that the RA test is a valuable aid in the diagnosis of hog cholera. Specimens to be tested should not be taken from swine which have recently received hog cholera antiserum.

SUMMARY

Additional data on the procedure of the resin agglutination (RA) test are presented. Comparative titrations with the RA inhibition and the serum neutralization (SN) tests were conducted on 43 serum samples containing neutralizing antibodies for vesicular stomatitis virus. There was good agreement between the two procedures. It is suggested that the RA inhibition test be used as a screening method in surveys of vesicular stomatitis. The RA test was used to detect hog cholera virus in materials from 12 outbreaks of diseases of swine. The serologic test proved to be a valuable aid in the diagnosis of hog cholera.
REFERENCES


Legend for fig.1—Correlation between serum neutralization (SN) and resin agglutination-inhibition (RAI) titers of 43 serums from bovine, swine and raccoons with antibodies to vesicular stomatitis. The SN titers are expressed as the log₁₀ of the number of chicken embryo LD₅₀ neutralized by one ml. of serum. The RAI titers are expressed as the log₂ of the reciprocal of the highest dilution of serum inhibiting four agglutinating units of virus. Each dot represents the titer of one serum sample with the two tests. The line of best fit is shown.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

S. H. McNutt, Chairman, Madison, Wisconsin; H. S. Cameron, Davis, California; C. N. Dale, Beltsville, Maryland; L. Davison, Okemos, Michigan; H. C. H. Kernkamp, St. Paul, Minnesota; F. P. Wilcox, Los Angeles, California (deceased).

The Committee on Transmissible Diseases of Swine wishes to express its appreciation to all those who have aided in furnishing information, but especially to the various State livestock sanitary officials. Also to be thanked is Dr. Charles Grey, Deputy Director of the State Experiment Stations Division, ARS, United States Department of Agriculture.

RESEARCH ON SWINE DISEASES

Funds which support studies for the advancement of knowledge are truly an investment. Because such funds rarely fail to give a good return, they are sound investments. Studies on small animals such as poultry require relatively small support and have been fairly adequate. The return has been the considerable advancements in the knowledge of poultry diseases, of which we are all justly proud. Investments in the study of diseases of large animals must be considerably greater to accomplish comparable results. For obvious reasons diseases of swine have been neglected more than the diseases of the other economically important animals. The public has not seen fit to invest reasonable sums in the investigation of swine diseases nor has the swine industry asked for such investments. More recently there has been a modest increase in the investment to study diseases of swine. In 1953 the United States Department of Agriculture employed $257,470 in research of such diseases—an additional $62,200 was supplied by states in cooperative studies with the United States Department of Agriculture. In 1958 the United States Department of Agriculture hopes to use $612,155 on swine disease studies and expects the States will supply about $102,240 additional in cooperative ventures. The United States Department of Agriculture is involved alone with studies on African swine fever, brucellosis, leptospirosis, and mycotic diseases. The United States Department of Agriculture is involved in cooperative studies with various states on atrophic rhinitis, hog cholera, swine erysipelas, vesicular diseases, mortality in young pigs, and miscellaneous diseases. Expenditures on state projects supported entirely by funds other than Federal grants are not included in the above. It appears that less than $1.00 of every $1,000.00 of swine income is invested in a study of swine diseases. Other industries would consider this totally inadequate. (See the note on the “Check-off bill, HR-7244, below.) Facts are stranger than fiction.


**GARBAGE FEEDING TO SWINE**

For control of swine diseases, especially vesicular exanthema, hog cholera, and trichinosis, it is necessary to cook all garbage fed to swine (see below). Yet about 200,000 swine are fed raw garbage in the United States. These few pigs jeopardize the entire swine industry. When it is considered that Iowa alone produced 22,000,000 hogs last year, it will be observed that a small number of garbage fed swine can endanger a large industry. Surely, such selfish principles are not those on which this country was developed.

The cooking of garbage that was fed to swine started in order to eradicate vesicular exanthema. The disease has not been recognized in over a year but it is still too early to predict that it has been eradicated, because some raw garbage is still fed. When eradication has been accomplished there will be a general relaxation of garbage cooking and even a repeal of the cooking laws. The cooking of garbage is also a necessity for hog cholera eradication.

**THE USE OF VIRULENT HOG CHOLERA VIRUS IN VACCINATION**

As of June 1, 1957, 14 States (South Carolina, Georgia, Alabama, Mississippi, Louisiana, Arkansas, Tennessee, Kentucky, Illinois, Wisconsin, New Mexico, Utah, North Dakota, and Montana) do not permit the use of virulent virus in the vaccination of hogs against cholera (the simultaneous method). This is because all hogs so vaccinated shed the virus for a time and are able to spread hog cholera to other swine. The use of virulent virus is an excellent means of spreading hog cholera. Two States (Oregon and Nevada) used no virulent virus in vaccination and two States (Michigan and California) used virulent virus in only one percent of the swine vaccinated. Of the swine vaccinated in other states with all types of vaccines, virulent virus was used in three to 25 percent of the swine vaccinated in 14 States (Washington, 10 percent; Idaho, 10 percent; Arizona, 25 percent; Wyoming, five percent; Colorado, 20 percent; Nebraska, 15 percent; Kansas, 20 percent; Oklahoma, three percent; Minnesota, 20 percent; Iowa, 25 percent; Missouri, 20 percent; North Carolina, 25 percent; New Jersey, 25 percent; Florida, 15 percent); 26 to 49 percent in five States (Indiana, 40 percent; Ohio, 30 percent; Virginia, 27 percent; Vermont, 30 percent; and South Dakota, 33 percent); 50 percent in eight States (Texas, Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, Delaware, Maryland); 65 to 75 percent in two States (New York, 65 percent, and West Virginia, 75 percent), and the situation was unknown in one State (Pennsylvania).

It is concluded that some states other than the 14 mentioned above will soon pass laws which prohibit the use of virulent virus in hog cholera vaccination. At the first session of the 85th Congress, March 13, 1957, a bill (H. R. 5933) "To control the preparation, distribution, importation and exportation of virulent hog cholera virus and for other purposes" was introduced by Mr. Grant. The bill was referred to the Committee on Agriculture. On Wednesday, July 24, 1957, the Subcommittee on Livestock and
Feed Grains of the Committee on Agriculture, House of Representatives, held a hearing on this bill in Room 1310, New House Office Building. The organizations appearing in favor of the bill were the United States Department of Agriculture, the American Meat Institute, the National Swine Growers Council, the American Farm Bureau Federation, the United States Livestock Sanitary Association, and the American Veterinary Medical Association. No organizations appeared against the bill. Yet a single individual (Mr. Charles Mills, Lewis, Delaware) appearing as a single swine grower against the bill was able to beat it single handed.

Truly "facts are stranger than fiction." Since then, various organizations have reconsidered their stand relative to the use of virulent virus and it is likely that all will reaffirm their stand in favor of outlawing such virus in vaccination. It is also very likely that Congress will pass such legislation.

The livestock check-off bill, HR-7244 which was to supply funds for all phases of the livestock industry was also beaten, this time by the very Congressmen who are supposed to represent livestock producers. Congressmen who represent livestock production to a much less extent were favorable. Truly, facts are stranger than fiction.

A request, in the form of a questionnaire went to livestock sanitary officials to furnish information on swine diseases. Reports were received from 35 states, Hawaii, and one Canadian province.

There was extreme uniformity on only one subject. All States agreed that the cooking of garbage had reduced disease in swine. Of course, the one Canadian province reported that the cooking of garbage was a must. In the face of this evidence, it is difficult to understand how any State can refuse to make it mandatory that all garbage fed to hogs be cooked; how any State can consider repeal of garbage cooking laws, or disregard its garbage cooking laws. All these things occur.

**FEEDER PIGS AND BREEDING STOCK**

The states that reported stated that 453,983 swine had moved interstate for purpose other than slaughter and show. All 28 states that reported considered the movement of feeder pigs a disease hazard. In four states, it was a major problem; in 20, a moderate problem; and in seven, a nuisance.

**HOG CHOLERA**

Hog cholera was reported to be prevalent last year in nine states. There was an increase in number of hog cholera outbreaks in only one state, no change in nine and a decrease in 17 states. All reporters agreed that traffic in swine was a main factor in cholera outbreaks. The failure to vaccinate hogs in traffic was also a cause of outbreaks. The decrease in number of hog cholera outbreaks was attributed to the following causes: (1) garbage cooking (10 states), (2) outlawing the use of virulent virus in vaccination (five states), (3) better sanitary measures (three states), (4) more vaccination (four states), and (5) the use of attenuated vaccines (two states).
SWINE ERYsipelas

Erysipelas was a problem in all major swine raising states—a serious problem in twelve. There was an increase of the disease in seven states, no change in 17, and a decrease in two while one state reported no swine erysipelas and another had not recognized the disease in 20 years. All reporters agreed that traffic in carrier or diseased hogs was a major factor in the spread of erysipelas while some believed that the use of live culture in vaccination was an additional factor. It has been pointed out to the Committee that there are areas which are relatively free of swine erysipelas. Hogs from these areas have little resistance to the disease. When such hogs, including feeder pigs, are moved to areas where the disease is endemic, large losses result.

ATROPHIC RHINITIS

Atrophic rhinitis continues about the same. It was reported a comprehensive problem in commercial herds in eight states, in pure-bred herds in 12 states, in “genetically bred” breeding stock in five, while it was not recognized as a serious problem in commercial herds in 22, or in pure-bred herds in 12 states. It was reported to be on the increase in seven states and no change in 12. All agreed that the disease was spread by traffic in swine and that infected or exposed breeding stock was a matter of grave concern.

LEPTOSPIROSIS

Twenty-six of 30 states believed that leptospirosis was not a serious problem in swine production. Nine of 19 states, reporting found that the disease was on the increase. Four out of 20 reporting found it a serious problem in pure-bred herds. The disease is spread by traffic in swine including breeding stock. One state reported no leptospirosis.

TRANSMISSIBLE GASTROENTERITIS

Transmissible gastroenteritis was a serious problem in four of 32 states reporting. It was on the increase in two with no change in 20. It was not decreasing anywhere. Several reporters pointed out the serious nature of this disease because no effective means of treatment or control are available.

ENTERITIS COMPLEX

This was considered a serious problem in nine out of 32 states reporting. It was on the increase in one state, with no change in 19 and a decrease in two.
RESPIRATORY DISEASES

Respiratory diseases were a serious problem in nine of 32 states. It was on the increase in one state, decreasing in two with no change in 16. Of special interest is that no part of the country escaped and that climate did not seem to be a factor.

EPERYTHROZOONOSIS

Eperythrozoonosis was reported to be a serious disease in only two states and to be on the increase in three. Of special interest is the fact that this disease is not recognized in several of the eastern and northeastern states. Is it possible that a part of the country escapes the disease?

BRUCELLOSIS

One state will not certify a herd of cattle as free from brucellosis unless all swine on the same farm are shown to be free of the disease. Two other states reported that swine brucellosis was a problem in the eradication of bovine brucellosis and three believed it to be a nuisance in bovine brucellosis. One state reported that brucellosis of swine had not been recognized within the state. Other than this the estimated percentage of infection was usually reported to be 0.1 percent to four percent. One state reported 25 percent infection and another 36 percent. Present belief on swine brucellosis does not support the contention that 35 to 36 percent of a large swine population could be infected. Hawaii reported a 50 percent herd infection. A search for the cause of such high incidence should be made. Fourteen states have a program dealing with swine brucellosis eradication. Eighteen states do not. The status of the others was not reported.

The Committee recommends the following:

1. That garbage cooking laws remain in force after the eradication of vesicular exanthema and that they continue to be vigorously enforced.
2. That the United States Livestock Sanitary Association favor the passage of some hog cholera virus law such as HR 5933 in Congress and that individual members work within their states for laws that will prohibit the use of fully virulent virus in the vaccination of swine.
3. That the United States Livestock Sanitary Association favor a “check-off” law providing funds from such check-off be employed to develop healthier swine as well as all other phases of the swine industry.

A copy of the Wisconsin law which prohibits the use of fully virulent virus is attached to this report as an example of such laws.
AN ACT to repeal and recreate 95.24 (1) of the statutes, relating to live hog cholera vaccine.

The people of the state of Wisconsin, represented in senate and assembly, do enact as follows:

95.24 (1) of the statutes is repealed and recreated to read:

95.24 (1) No person shall have in his possession or furnish to another any vaccine or other substance capable of producing hog cholera, except that any such substance may be in the possession of a biological laboratory inspected and licensed by the federal government or a person who has written approval from the department for its experimental use.

______________________________
Speaker of the Assembly.

______________________________
President of the Senate.

This act originated in the Assembly.

______________________________
Chief Clerk.

Approved, 1957.

______________________________
Governor.
FURTHER STUDIES ON ENZOOTIC VESICULAR STOMATITIS

R. P. HANSON and LARS KARSTAD

Vesicular stomatitis in the United States occurs in two forms, epizootic and enzootic. The epizootic form is seen as a rapidly spreading disease affecting thousands of cattle and horses over wide areas. Outbreaks of this nature have been seen in Wisconsin at intervals of about 10 years, the last occurring in 1949 (2). Less extensive epizootics have occurred at more frequent intervals in some of the southern states.

The enzootic is characterized by scattered cases occurring each summer in the areas involved and its frequent appearance in swine. The regions in which enzootic vesicular stomatitis is known to occur are situated in the southeastern United States (8) and in Mexico, Columbia and Venezuela (3).

Enzootic vesicular stomatitis varies from the epizootic form in several basic ways. First, there is the annual occurrence of the enzootic as opposed to the erratic appearance of the epizootic form. The epizootic is more dramatic than the enzootic in its involvement of greater numbers of animals over a wider area, in its rapid spread, and often in its higher morbidity in affected herds. Cattle and horses are the chief species infected in epizootics, while swine are frequently infected in enzootic areas. Deer, raccoon and bobcats have been found to be hosts for the virus in enzootic areas (6), while these species have not yet been observed to be involved in epizootics.

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This study is part of an epizootiological investigation being carried out by the Wisconsin Agricultural Experiment Station with the support of the Agricultural Research Service of the United States Department of Agriculture.

Both forms of the disease agree in certain respects. The infection usually appears in animals having access to wet, wooded pastures. It may involve animals in pastures bordering a stream or swamp, while others a short distance away in open, well-drained fields may escape. The spread of infection generally cannot be related in any way to the movements of animals or contaminated materials. At the time outbreaks occur biting insects, mosquitos, gnats and horseflies are often numerous. This has encouraged the hypothesis that such insects may act as vectors of the virus (9), but up to the present natural transmission by insects has not been confirmed. Whether enzootic or epizootic, the disease is seasonal in nature, appearing usually during the summer and disappearing at about the time of killing frosts in the fall. In the southern states it is difficult to relate the cessation of summer outbreaks to fall frosts, since these do not occur until much later.

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Several important questions regarding the epizootiology of vesicular stomatitis remain unanswered. What accounts for its seasonal occurrence? What maintains the virus over winter? What are the transmission mechanisms involved? And finally, why is the enzootic area so restricted geographically?

In our attempt to answer these questions we have made use of several basic approaches. We began by the collection of data from the field. This took the form of questioning residents of enzootic and epizootic regions and of conducting serological surveys for evidence of prior infections in wildlife, domestic animal and human populations. The second part of our study was conducted in the laboratory. Materials collected in the field were processed for viral isolation. Experimental infections were initiated to study the course of infections in the new hosts detected by serology. Experiments were set up to detect the persistence of virus in animals suspected of acting as reservoirs for the virus. Finally, a study of natural transmission of the disease was set up within the enzootic region in southern Georgia. This makes use of a partially controlled area in the sense that attempts are made to restrict the movement of wild animal hosts of the virus while studying the natural spread of infection among these animals.

Based on information collected in southeastern Georgia, three criteria have been established for determining whether or not vesicular stomatitis is present in an area in enzootic proportions. An area may be designated enzootic if one or more of the following apply:

1. Fifty percent or more of the cattle and swine, regardless of age, carry virus neutralizing antibodies against vesicular stomatitis virus.
2. Virus neutralizing antibodies are found in wild animals.
3. Vesicular stomatitis has been diagnosed by virus isolation or by serology for a number of successive years.

Replies to questions regarding the probable duration of vesicular stomatitis as a seasonally occurring disease of livestock in the southeastern Atlantic Coastal Plain indicate that disease conditions clinically resembling vesicular stomatitis have appeared in some areas each year as long as the oldest residents can remember. It seems probable that the recent nation-wide campaign to eradicate vesicular exanthema has aided materially in making us aware of the widespread occurrence of vesicular stomatitis in swine.

Surveys for the presence of virus neutralizing antibodies in the sera of livestock, wildlife and man have been very productive in adding to our knowledge of the normal host range of the virus and the distribution of the disease as an enzootic. Results from southeast Georgia indicate that approximately 45 percent of the domestic swine, 50 percent of the cattle and 80 percent or more of the horses and mules carry antibodies against New Jersey type vesicular stomatitis virus. Similarly, neutralizing antibodies were found in about 75 percent of the feral swine, 45 percent of the raccoon and 60 percent of the deer.
Serum neutralization titers against the New Jersey serotype, vesicular stomatitis virus have been found in a number of birds which inhabit the Carolina and Georgia sea coasts. Efforts are being made to rule out the possibility that the observed virus neutralization is due to some non-specific substance in the sera of these birds.

In the examination of human sera received by the State Public Health Laboratory at Waycross in southeast Georgia approximately 30 percent were found to be carrying neutralizing antibodies against New Jersey type vesicular stomatitis virus. Information gained by observation of vesicular stomatitis virus infections among laboratory workers and diagnostic personnel indicates that infections are typically characterized by an influenza-like syndrome (5). Symptoms most often observed are fever, headache, chills, muscular pain and general malaise. These persist for one to three days after which the patient makes an uneventful recovery. It is easily understood how illness of this type may be misdiagnosed for influenza, malaria or other transient febrile infections. Attempts to confirm a clinical diagnosis are discontinued when the patient makes a rapid and complete recovery. Through the cooperation of the Georgia Department of Public Health paired sera are being collected from febrile illnesses among residents of the enzootic area, so that recent infections are

![Prevalence of Vesicular Stomatitis in South Carolina](image_url)

**Fig. 1.** Prevalence of Vesicular Stomatitis in South Carolina.

Broken line indicates position of the Fall Line. Solid line is the upper boundary of enzootic area based upon interviews conducted by the office of the State-Federal Veterinarian of South Carolina.

Counties in which swine serums contained antibodies are marked by a cross +. Counties in which serums were negative are marked by a dash —.
can be recognized and data collected. It is important to know the relationships which exist between the occurrence of vesicular stomatitis virus infection in humans and their appearance in other animals. Detailed histories on cases of human infection may aid greatly in detecting the sources of infection and the transmission mechanisms involved.

A survey for vesicular stomatitis virus neutralizing antibodies in the sera of mature native hogs has aided in the delimitation of the enzootic area in South Carolina. Serum samples were collected throughout the State by personnel of the State-Federal Livestock Disease Eradication Program, efforts being made to sample as many counties as possible. In most areas data gathered by the testing of these sera was supplemented by information collected by farm to farm questioning of livestock owners and by consultation with practicing veterinarians regarding the previous occurrence of vesicular diseases. The results of these surveys indicate that the vesicular stomatitis enzootic area is restricted to the Coastal Plain and that the Fall Line which separates the Coastal Plain and Piedmont regions may be said to form the upper boundary of the enzootic area (Figs. 1 and 2). This belt of enzootic vesicular stomatitis appears to extend into the Coastal Plains of North Carolina and Georgia.

This year vesicular stomatitis appeared in cattle in two counties in the Piedmont region of South Carolina. Careful investigations conducted by State-Federal regulatory personnel seem to indicate that these outbreaks represent epizootic extensions from the enzootic area, since the disease appeared mainly in cattle, older age groups were often infected, and veterinarians and livestock owners had not observed the condition in previous years. These characteristics of the outbreaks agree with observations on epizootics in other areas.

It is felt that investigations similar to those carried out in South Carolina this year would serve to determine the geographic limits of enzootic areas in other states. This information may be of considerable value in formulating the control procedures to be followed in outbreaks of vesicular disease during the time required for diagnosis.

Attempts to isolate virus from animal tissues collected in the field, except in the case of livestock, have been unsuccessful. A wide variety of materials have been processed, emphasis being placed on the detection of virus in the tissues of the recognized hosts of vesicular stomatitis and from lower forms of life in direct or indirect association with these species. This includes parasites and animals which may serve as items of diet for the recognized viral hosts.

Studies of experimentally induced infections in the newly recognized wild animal hosts (deer (seven) and raccoon (four)) indicate that these species are subject to acute, short term infections, followed in uncomplicated cases by complete recovery and high levels of virus neutralizing antibodies. We have failed to demonstrate the persistence of a carrier state following experimental infections in these species as well as in cattle and swine.
A number of experiments have been conducted in attempting to detect long-term virus survival in lower animals suspected of acting as reservoirs of infection (4). Earthworms, a major item of diet for hogs during warm damp weather, were exposed to vesicular stomatitis virus by a variety of routes. Virus was inactivated quite rapidly in the earthworm. Frogs (Rana
STUDIES ON ENZOOTIC VESICULAR STOMATITIS

Another item of diet for hogs and raccoon, were infected by subcutaneous inoculation and oral administration. Vesicular stomatitis virus was recovered from the lungs, and in one instance from the skin of the hibernating animals for up to five weeks after exposure. While evidence of virus multiplication was not obtained, these experiments did indicate a potential role for cold-blooded animals in carrying the virus over winter while hibernating.

A number of serological techniques have been developed and used during the course of serological surveys. Serum neutralization of virus detected by the inoculation of embryonated eggs was used as the standard procedure and this has been used to evaluate the results of tests conducted by resin agglutination (10) and virus neutralization in cell cultures.

The resin agglutination test developed by Segre for the detection of specific viral antigens was adapted by Segre and Nieto for use as an inhibition test in the detection of antibodies against vesicular stomatitis. The results of these tests correspond to a reasonable degree with those of virus neutralization tests using the same sera and antigens. However, it was found that the resin agglutination test for vesicular stomatitis was far less type specific than the neutralization test, in that cross reactions did occur between the New Jersey and Indiana serotypes. Also it was found that the reagents used in the test were unstable requiring daily preparation.

Serum neutralization tests were conducted in bovine kidney epithelial cell cultures grown in one ml. cups in plastic plates. These were sterilized with alcohol and discarded after use. Tests are underway at present to determine the feasibility of using cell cultures instead of chicken embryos for routine vesicular stomatitis serological survey work.

A new technique for the field collection, preservation, shipping and storage of serum samples was developed using small discs of white absorbent paper (1). Paper discs, dried after saturation with serum, require no refrigeration and can be very economically shipped and stored. Broth eluates prepared from these dried samples were tested in the usual manner by virus neutralization in chicken embryos.

In an effort to control some of the variables encountered in an epizootiological study of this type we have set up the "controlled study area" in southeast Georgia, where natural transmission of vesicular stomatitis among sylvan hosts can be observed. A peninsula jutting out into the Altamaha River near Brunswick, Georgia has been isolated by the construction of a double hog wire fence across its neck. This portion of Champney Island comprises about 22 acres of swampy wooded land. Work on this peninsula was commenced in May, 1957, when the fence was erected and the initial status of the flora and fauna were surveyed. Feral pigs, captured on the area at this time bore snout lesions suggestive of vesicular stomatitis. These pigs gave evidence of current infection by rising titers of neutralizing antibody against New Jersey type vesicular stomatitis virus. All pigs present on the peninsula at this time were tested by the serum neutralization test. Those which gave evidence of antibodies against vesicular stomatitis virus were removed. Apparently
healthy pigs which did not carry neutralizing antibodies were retained to be used subsequently as sentinels to detect virus activity in the area. To these were added feral pigs trapped on other portions of Champney Island. All pigs were blood tested and ear tagged before release. In the event that their tests gave evidence of previous infection with vesicular stomatitis virus, they were recaptured and removed from the area. By this procedure it was thought possible to build up a population of vesicular stomatitis susceptible pigs which might be studied in their natural habitat and which would be used as sentinels to detect the continuing presence of vesicular stomatitis in the area.

Such a high percentage of the hogs trapped had serum neutralizing antibodies that trapping and stocking operations conducted throughout the summer failed to give us the desired number of susceptible pigs. About two-thirds of the pigs under 50 pounds body weight were positive to the neutralization test when first captured. Pigs over 50 pounds were not retained for testing as it was assumed by reason of past experience that the majority of these older hogs would have had previous virus exposure. Of the younger hogs which were negative to the test when captured, many became infected on the study area, thus giving evidence of continuing virus activity throughout the summer. Lesions were not often observed but infection was diagnosed by the appearance of neutralizing antibodies. Many of the pigs were but a few weeks old when captured. Assuming that colostral antibodies might account for antibody titers in these very young animals, several were retained on the study area. Some of these became negative on subsequent tests, indicating the disappearance of colostral antibodies, only to become positive to tests later on, indicating exposure to the virus present on the study area.

We may enumerate the main points of the information gained by this study as follows:

1. An opportunity was afforded to study the habits of feral swine under natural conditions.
2. Observations were made on the ecological relationships of the hogs with the other animals of the area.
3. Information on the frequency of infection of hogs with vesicular stomatitis virus on the study area and on the other portions of Champney Island was obtained.
4. Normal serological trends were observed. Pigs at birth received colostral antibodies against vesicular stomatitis virus from their dams. These antibody levels receded during the first weeks of life until exposure to the virus stimulated an active immune response.

The very high frequency of vesicular stomatitis infections in the hogs on Champney Island has resulted in the failure of our attempt to build up a population of vesicular stomatitis susceptible feral pigs. Our next step will be to remove all feral pigs presently occupying the peninsula and substitute a group of young domestic hogs known to be susceptible to the infection. These pigs will be stocked during the winter when virus activity in the area is slight
or absent. They will act as sentinels to detect the presence of virus activity during the winter, or its absence and resumption of activity in the spring. In the event that our findings should indicate occasional infections of swine throughout the winter, it would then no longer be necessary to include the hypothesis of a long-term reservoir for the virus in attempting to explain the continued survival of vesicular stomatitis virus in the southeast.

REFERENCES

VESICULAR STOMATITIS IMMUNIZATION WITH INACTIVATED VACCINES OF CHICKEN EMBRYO ORIGIN*

A. A. HOLBROOK, D.V.M., and J. N. GELETA, D.M.V., M.S.

The economic losses caused by vesicular stomatitis (VS) and its similarity to foot-and-mouth disease and vesicular exanthema are ample reasons for continued studies to determine the best methods for its control and possible eradication in the future.

Hanson and Karstad (1) reported that New Jersey type VS is found as an enzootic disease in most of the lower coastal plains in the southeastern United States and as an epizootic disease in parts of northern and western United States. VS appears in the enzootic area each May or June and disappears with the first killing frost in the fall. This limits the time of spread to only five or six months.

Our objective in this work was to develop and test an inactive vaccine effective in protecting animals, especially swine, against VS. An efficient vaccine used just before the usual outbreak in May or June might be the means of breaking the cycle or otherwise aiding in the control of this disease. Swine were chosen as the species on which to test the vaccines, since the first cases each year are seen in swine. Egg-grown virus was chosen because of the economy of production, safety, and ease with which large quantities could be produced if needed.

Strain of the Virus. The egg-adapted New Jersey type Concan strain of VS was employed in this study. This highly antigenic strain used at our laboratory in a great deal of typing and experimental work was isolated from the dental pad of a cow involved in an original field outbreak at Concan, Texas. Tested serologically and immunologically, it was found to be New Jersey-type VS and was designated N.J. Concan FS-2, June 1949 VS. The original virus sample was kept frozen in a dry-ice chest from the date received until the first cattle passage on June 11, 1951. Subsequently, two more passages in cattle and three in developing chicken embryos were made.

Infected amnioallantoic fluids harvested from the last egg passage of the virus, tested for sterility in thioglycollate medium, and maintained at −60°C. in sealed glass bottles were used as a seed virus for inoculation of the embryos. This material when tested contained $10^{8.9}$ embryo lethal doses for 0.1-ml. inoculum via the allantoic cavity in eight-day-old embryos.

Method of Inoculation and Virus Production. In an effort to obtain as high infective titers as possible and to ascertain the portion of the embryonated eggs which contained the highest concentration of the virus, seven-...
eight-, nine-, 10-, and 11-day embryos were tested for their ability to support the growth of the egg-adapted VS virus. Dead embryos, chorioallantoic membranes, yolk sacs, and amnioallantoic fluids were collected aseptically from five inoculated eggs of each group. After preparation, these materials were titrated in eggs and by the complement-fixation test. In each age group there was found to be sufficient virus for vaccine production.

In these investigations eight- or nine-day-old embryonated eggs were used. One hundred and eighty embryonated eggs were inoculated for preparation of vaccine material. After disinfection of the eggs with tincture of iodine, a Vibra Graver was used to drill a small hole in the center of the air space, cutting through the shell but leaving the shell membrane intact. With a 1-ml. tuberculin syringe and a three-quarter-inch 26-gauge needle, 0.1 ml. of infected amnioallantoic fluid was injected vertically into the allantoic cavity. The eggs were sealed with melted paraffin and reincubated at a reduced temperature of 35° to 36°C. All deaths of embryos occurring before 16 hours were attributed to injury, and the eggs were discarded. The remaining eggs, in which the embryos died between 16 and 28 hours postinoculation, were stored overnight at 4° C. The next morning the amnioallantoic fluids were aspirated with sterile pipettes and pooled. The contents of each egg were then poured into a petri dish; chorioallantoic membranes, embryos, and yolk sacs were aseptically collected.

Preparation of Vaccines. The virus-laden amnioallantoic fluids and the pooled contents of embryonic tissues were milled together in an Eppenbach colloid mill for six minutes. The homogenized emulsion was then centrifuged for 10 minutes at approximately 2,500 r.p.m. in an International centrifuge to remove gross tissue fragments and pooled in a single container before further processing. At this time a sample was removed for bacteriological and serological tests. After a sterility test was performed on blood agar plates and in thioglycollate medium, with negative results, the virus content of each batch was determined by titration in embryonated eggs and an antigen titration was run by complement fixation, using homologous hyperimmune guinea pig serum. Suspensions which showed a LD$_{50}$ virus titer less than 10$^{-8}$ were considered as not suitable for production of the vaccine.

Crystal Violet Heat-Inactivated Vaccine. Eighty parts of prepared embryonic tissue suspensions were thoroughly mixed with 20 parts of a solution of crystal violet in glycerin. This solution (2), as used in the preparation of hog cholera vaccine, contained one part crystal violet in 400 parts of glycerin. The mixture was incubated at 37°C. Samples were taken regularly and tested for infectivity on guinea pigs and in eight-day-old embryonated eggs as long as the samples proved infective. Shahan (3) found that crystal violet used in this manner inactivated VS virus added to defibrinated blood in two or three days. The first batches prepared in this work using egg-propagated virus required nine to 12 days for complete inactivation. Later when larger batches of vaccine were made, more time was required for inactivation. One batch of two liters required 21 days. The complement-fixation titer con-
tinually decreased with incubation time. After inactivation, the vaccine was stored in a commercial refrigerator at $4^\circ$C.

**Crystal Violet Beta-Propiolactone-Inactivated Vaccine.** The suspension of infectious material containing chorioallantoic membranes, yolk sacs, embryos, and amnioallantoic fluids, after centrifugation at 2,500 r.p.m. for 10 minutes, mixed immediately with beta-propiolactone in a final concentration of 0.1 percent. The pH of the mixture was adjusted to 8.2 with one molar disodium acid phosphate before beta-propiolactone was added. The technique of Lo-Grippo and Hartman (4) for the preparation of a water solution of beta-propiolactone was used. A 10 percent solution was made in glass-stoppered bottles with sterile distilled water at $4^\circ$C. and kept in an ice bath until used. After one hour of incubation at $37^\circ$C. the pH of the mixture was adjusted to 7.2 with diammonium acid phosphate. To 80 parts of treated virus suspension, 20 parts of a solution of crystal violet in glycerin were added, and the experimental vaccine was then stored at $4^\circ$C.

**Sterility, Infectivity, and Titration Tests on Vaccines.** Samples from each lot of processed experimental vaccine were examined for sterility on appropriate bacteriological media. Blood agar plates, nutrient agar slants, and thioglycollate media were inoculated with sampled material and incubated for 24-48 hours at $37^\circ$C. To test for active virus, five normal guinea pigs were inoculated with the vaccine by intradermal tunneling into the plantar pads. All guinea pigs were periodically observed for the development of lesions. Concurrently, 0.2 ml. of the tested vaccine material in tenfold dilutions was injected into five eight-day-old embryonated eggs via the allantoic sac. All eggs were incubated at $36^\circ$C. and held for five days before discarding.

The titration studies were conducted on vaccine material centrifuged at 2,000 r.p.m. for 10 minutes. The complement-fixation antigen present in supernatant fluid, in twofold dilutions of 1:2 to 1:512, was tested against strain-specific hyperimmune guinea pig serum. The fixation and lysis periods were 20 and 30 minutes, respectively, at $37^\circ$C.

**Serum-Neutralization Test.** All prevaccination and postvaccination serums were tested for the presence of specific virus-neutralizing antibodies by inoculation into eight-day-old embryos 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 serums inactivated at $56^\circ$C. for 30 minutes. Penicillin and streptomycin were added to the mixture in quantities sufficient to make a final concentration of 2,500 IU of each per milliliter of inoculum. After incubation in a water bath at $25^\circ$C. for 30 minutes, the mixtures were inoculated into the allantoic cavity, using five eggs per dilution. Incubation of the eggs was continued at $36^\circ$C., and death of all embryos after 16 hours recorded. Serum-neutralization titers were expressed in chicken-embryo LD$_{50}$ units and were calculated on the difference between the prevaccination and postvaccination serums.
Method of Vaccination and Challenge. The swine were crossbreeds secured from the Animal Husbandry Research Division, ARS, Beltsville, Maryland. All vaccinations were made subcutaneously on the inner surface of the fold of the flank. 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 rubbing a thick paste of vesicle coverings, previously harvested from New Jersey Concain-infected swine, into the scarified areas. Normal animals were used each time to check the virulence of the inoculum. Strict quarantine and disinfection procedures were maintained during all challenges.

Test of the Immunizing Capacity of Inactivated Egg-Propagated Vesicular Stomatitis Vaccines. Studies regarding immunizing power of vaccines and routes of inoculation were carried on with the first two experimental batches of vaccines. In these tests the administered dose and the immunity response to subcutaneous, intraperitoneal, and intramuscular inoculation were compared in guinea pigs. Three weeks after vaccination the guinea pigs were challenged by scarification and serums were collected for detection of protective antibodies by the serum-neutralization test. The subcutaneous route of inoculation gave the most protection and was adopted as the routine method of vaccination. One of the first considerations concerning the inactivated vaccines was whether immunity could be produced in swine. The vaccine (No. 3) used was made in the described manner, with inactivation occurring in 14 days at 37°C. Two groups of normal swine were used. The swine in one group weighed approximately 125 lb., and the ones in the other group weighed approximately 300 lb. Five swine of each weight were vaccinated subcutaneously with five ml. One week later these 10 swine and an additional five in each group were vaccinated with five ml. in the same manner. Three weeks after the last vaccination the 20 vaccinated swine and five controls from each group were challenged. The results of this test are outlined in Table 1. Both the single- and double-vaccinated swine were immune, but the serum-neutralization titers showed considerable differences between individual animals. The higher titers were found in the swine receiving a double vaccination. From this experiment it is readily deduced that immunity was produced by the vaccine, but the minimal dose required to produce immunity, or the duration of immunity, was not determined.

A new and larger batch of virus was prepared. A portion was treated with crystal violet in glycerin (vaccine four) and placed in the incubator at 37°C. for inactivation. To the other portion 0.1 percent beta-propiolactone was added, and the mixture incubated at room temperature for two hours; the pH was adjusted and the mixture stored at 4°C. Both vaccines were tested at regular intervals for infectivity. The first portion required 21 days’ exposure at 37°C. to become completely inactivated. The portion containing beta-propiolactone still had live virus in it after four days’ storage but was inactive on the fifth day.

One hundred swine were divided into two groups of 50 each. The heat-inactivated vaccine was used in one and the beta-propiolactone-inactivated
TABLE 1

Immunization of Swine Against Vesicular Stomatitis With Heat-Inactivated Egg-Propagated Virus Vaccine 3

<table>
<thead>
<tr>
<th>Number of Swine</th>
<th>Weight (lb.)</th>
<th>Amount of Vaccine Per Injection (ml.)</th>
<th>Number of Subcutaneous Injections</th>
<th>Challenge Scarification of Snout With Paste*</th>
<th>LD 50 Serum Neutralization Titers*</th>
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<tbody>
<tr>
<td>5</td>
<td>125</td>
<td>5</td>
<td>1</td>
<td>0/5†</td>
<td>0.38</td>
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<td>1.01</td>
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</tr>
<tr>
<td>5</td>
<td>125</td>
<td>Controls (not vaccinated)</td>
<td>5/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>Controls (not vaccinated)</td>
<td>5/5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three weeks after last injection.
† Numerator indicates number of swine positive; denominator indicates number of swine in group.

in the other. Each group was divided into five lots of 10 pigs each. Four lots of each group were vaccinated with 1, 2.5, 5, and 10 ml. respectively, and one lot was held as control.

Table 2 shows the immunity obtained by use of the heat-inactivated vaccine. At one month after vaccination, two animals from each of the five lots were challenged. None of the animals showed solid immunity, but one of the swine vaccinated with 2.5 ml. and both of the swine vaccinated with 10 ml. did show some immunity. After observation for 10 days the swine were destroyed.

Five weeks after the first vaccination, all the remaining swine were revaccinated from the same batch of vaccine and with the same dosage. Challenged at one month as before, the swine vaccinated with one ml. had elevated temperatures and developed lesions, as did the controls. Some pro-
**TABLE 2**

*Immunization of Swine Against Vesicular Stomatitis With Heat-Inactivated Egg-Propagated Virus Vaccine*

<table>
<thead>
<tr>
<th>Amount of Vaccine Per Subcutaneous Injection (ml.)</th>
<th>Single Vaccination</th>
<th>Double Vaccination*</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Challenge Scarcification of Snout With Paste†</td>
<td>LD 50 Serum Neutralization Titer†</td>
</tr>
<tr>
<td>1</td>
<td>4+‡</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>4+</td>
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<tr>
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<td>2+</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>2.00</td>
</tr>
<tr>
<td>5</td>
<td>Died</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1.00</td>
</tr>
<tr>
<td>Controls</td>
<td>4+</td>
<td>—</td>
</tr>
<tr>
<td>(Not vaccinated)</td>
<td>4+</td>
<td>—</td>
</tr>
</tbody>
</table>

* Five-week interval between vaccinations.
† One month after last vaccination.
‡ Amount of extension of lesion.

Protection was shown by the swine vaccinated with five ml.; the swine vaccinated with 2.5 and 10 ml. were negative. In this particular experiment the animals vaccinated with 2.5 ml. gave more protection than those vaccinated with five ml. This can probably be explained only by individual differences, as in most other instances the more vaccine used the greater was the protection demonstrated.

In Table 3 are shown the results of vaccination with the beta-propiolactone-inactivated vaccine. All the vaccinated swine were immune at one month, but at two months immunity was shown only in the swine vaccinated with 10 ml., and only one of them showed complete protection. At three months none of the vaccinated swine were proved completely immune but a partial immunity was shown by those vaccinated with 10 ml. The serum-neutralization titers continually decreased with time, although individual differences occurred with the same amount of vaccine.

**DISCUSSION**

We have prepared a vaccine which, under experimental conditions, gave a complete immunity in swine for one month. The largest amount of vaccine used (10 ml.) gave a partial immunity for three months. The second vaccine used in swine was one which required 21 days for inactivation and had a low complement-fixation titer. It showed very little protection with one vaccination, but after the second vaccination a greater protection. It is con-
<table>
<thead>
<tr>
<th>Immunization Amount of Vaccine Per Subcutaneous Injection (mL)</th>
<th>1-Month</th>
<th>Challenge* 2-Month</th>
<th>3-Month</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Scarification of Snout With Paste</td>
<td>LD 50 Serum Neutralization Titer</td>
<td>Scarification of Snout With Paste</td>
</tr>
<tr>
<td>1</td>
<td>Negative</td>
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<tr>
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<td>Negative</td>
<td>3.50</td>
<td>2.75</td>
</tr>
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</table>

**Controls** (Not vaccinated)

4+ 4+ 4+ 4+

* On each challenge 2 different swine were taken from each group in which the different amounts of vaccine had been used.

† Amount of extension of lesion.
ceivable that an increase in the dosage and a second vaccination may give enough protection to prevent spread of VS, but this can only be determined by future work.

While no exhaustive attempts have been made to evaluate the relative merits of the heat-inactivated vaccine as compared with the beta-propiolactone-inactivated vaccine, the evidence indicates a margin of superiority for the latter. This, considered with the more rapid inactivation of the virus suspension, leads us to believe it is the more desirable of the two.

In the preparation of all these vaccines, it was found that the time for complete inactivation varied a great deal, regardless of the method of inactivation. It was postulated that the lipid material on the yolk sacs protected the virus. When a new suspension was made as before, but without the yolk sacs, the virus was completely inactivated by the addition of 0.1 percent beta-propiolactone in one hour. The crystal violet in glycerin maintained the sterility in all vaccines and did not interfere with the complement-fixation test. No ill effects were noted when the inactivated vaccines were injected into embryonating eggs, guinea pigs, or swine.

SUMMARY

Crystal violet vaccines of chicken embryo origin which were inactivated by incubation at 37°C and by the addition of beta-propiolactone were tested in swine. The beta-propiolactone-inactivated vaccine proved to be the more efficient and gave complete protection for one month against challenge with vesicular stomatitis virus when 1-, 2.5-, 5-, and 10-ml doses were used, and gave a partial protection for three months when 10-ml doses were used. The vaccine inactivated with heat produced less immunity, but when used in a second vaccination it prolonged the immunity.

ACKNOWLEDGMENT

The authors are pleased to acknowledge the valuable advice of Dr. O. L. Osteen and Dr. L. O. Mott, of the Animal Disease and Parasite Research Division, in connection with the studies here reported.

REFERENCES
2. REPORT of the Chief of the Bureau of Animal Industry: ARA, United States Department of Agriculture, 22, 1944.
REPORT OF THE COMMITTEE ON VESICULAR DISEASES

R. A. BANKOWSKI, Chairman, Davis, California; F. CAMARGO, N., Obregon, Mexico D. F.; J. W. MANN, Atlanta, Georgia; L. O. MOTT, Beltsville, Maryland; F. J. MULHERN, Falls Church, Virginia; J. TRAUM, Greenport, Long Island, New York; K. F. WELLS, Ottawa, Ontario.

In reviewing the status of vesicular diseases of animals in the United States, we recognize that foot-and-mouth disease has not been reported since 1929. The last case of vesicular exanthema was reported in November, 1956. As you are aware, the program for these diseases is inspection, quarantine, slaughter, cleaning and disinfecting, and intrastate and interstate control over movement of susceptible animals and heat treatment of all garbage fed to swine.

In contrast, vesicular stomatitis is believed to have been present in this country during Civil War days. Due to its general past history, the disease usually runs a mild course; hence, little has been done about it. It is now well established in certain areas of the country.

VESICULAR EXANTHEMA

The status of the eradication program continues to be favorable. No cases have been reported during the past 12 months. With the exception of New Jersey, California, and South Carolina, it has been over three years since infection was reported in any of the 42 states that had the disease during 1952 and 1955. The last infection was reported on three premises in New Jersey during August, October, and November, 1956. These same herds were involved during 1952 and 1954. Eradication measures were not carried out following the 1952 and 1954 outbreaks. In 1956 all of the infected and exposed swine were slaughtered and all the pork from those that passed for food was specially processed. The infection prior to the New Jersey outbreaks was in California during November of 1955.

A garbage-cooking law was passed in New Jersey that will become effective January 1, 1958. A satisfactory garbage-cooking program was established in Texas. At the present time the majority of the opposition to garbage cooking is among feeders from Connecticut and Massachusetts.

A bill to repeal the garbage-cooking law in Massachusetts was sent to a special committee for further study. The committee is to (1) evaluate the need to continue to have such a law, (2) study the effects of the law, (3) study the degree of enforcement in Massachusetts and other states, and (4) see if any subsidies need to be given to feeders to compensate them for the purchase and maintenance of equipment needed to comply with the law.

A proposed garbage-cooking law sponsored by Connecticut health authorities was defeated. This is the only state that does not have a garbage-cooking
law. Recently, several feeders, who previously opposed garbage cooking in
the state, have expressed interest in purchasing garbage-cooking equipment.

In some states a few of the smaller feeders are not complying with the
state laws. No attempt is being made in those states to compel feeders to
comply with the laws even though cases have been repeatedly brought to
the proper state official's attention. This is an alarming situation.

VESICULAR DISEASES

VESICULAR STOMATITIS

Last year's committee report recommended that this disease be made re-
portable. It would be highly advantageous if we could determine the true
extent of infection in this country.

Like most diseases that are allowed to go on unhampered, we see different
forms. In some cases the entire herd has been involved; usually only a few
animals show outward signs of the disease. This year more cases have been
reported with teat lesions. In a few herds the teat lesions became quite
prevalent and there was a drastic reduction in milk production for varying
periods.

The condition has frequently been observed in swampy, wooded, and
generally in lowlands; however, this year and in other years it has been ob-
served in the mountainous areas of West Virginia and Virginia. Generally
only a few cases are found in a herd and the next case is reported five to 10
miles away. However, in southeastern Oklahoma and southwestern Arkansas,
over 300 herds were involved in four counties.

The recommended procedure is to prevent movements of animals from
infected premises until it is determined that the disease within the herd has
run its course. Where a diagnosis of VS is made it should be reported to
state public health officials. In the Vesicular Disease Committee report of
the 59th annual United States Livestock Sanitary Association meeting in New
Orleans, this Committee states "The term mycotic stomatitis has been so in-
discriminately and loosely applied as to include almost any form of stomatitis
not showing vesiculation at the time of examination. Many such cases may
well have been vesicular in nature in earlier stages". This past year some
diagnosticians have observed in the same herd, both a vesicular condition and
a non-vesicular stomatitis which they have called mycotic stomatitis. How-
ever, serology showed that in these herds animals having either type of mouth
lesions were positive for vesicular stomatitis, New Jersey type. In other in-
stances, when the non-vesicular stomatitis was observed alone, the serology
was positive, indicating that whenever a non-vesicular stomatitis is present,
it is not possible to diagnose the condition without serological tests. It
should be pointed out, however, that some cases of so-called mycotic stomatitis
were completely negative for both types of vesicular stomatitis as well as six
type of foot-and-mouth disease.
GOALS FOR V. E. ERADICATION PROGRAM

2. Defeat of bill to repeal garbage-cooking law in Massachusetts.
3. Strict enforcement of all state garbage-cooking laws.
4. Quarantine all swine being fed raw garbage.
5. Require that all raw garbage-fed swine be slaughtered and specially processed.
6. Recommend that state garbage-cooking laws be amended to state as their justification the prevention of not only vesicular exanthema but other livestock diseases that can be introduced or spread through the feeding of raw garbage, such as foot-and-mouth disease, hog cholera, trichinosis, tuberculosis, erysipelas, salmonellosis, and some streptococcal infections.

GOALS FOR VESICULAR STomatITIS

1. Bring to attention of all practitioners the need to report suspect cases.
2. Prevent the movement of animals from infected premises until it is determined that the disease within a herd has run its course.
3. Efforts should be made to diagnose condition by serological test on each premises from which it is reported.
4. Determine areas in country in which vesicular stomatitis is enzootic.
5. Determine why it is enzootic in certain areas.
6. Determine economic effects of disease on livestock in the area.
7. Etiological studies should be made of so-called mycotic stomatitis.

THE INCIDENCE OF FOOT-AND-MOUTH DISEASE IN FOREIGN COUNTRIES

Reports from the International Office of Epizootics, and other sources indicate that a new wave of foot-and-mouth disease is sweeping over Europe. Vaccines are used to assist in control of the disease in practically all countries in Europe, with the exception of England where the slaughter method is depended upon.

New Farms Reported to be Infected With Foot-and-Mouth Disease January to Date (1957)

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<th>Country</th>
<th>No. of Infected Farms</th>
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<tr>
<td>Denmark</td>
<td>45</td>
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<tr>
<td>France</td>
<td>89,929</td>
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<td>Spain</td>
<td>143</td>
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<tr>
<td>Switzerland</td>
<td>120</td>
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VESICULAR DISEASES

Latest reports indicate that Turkey had the most severe outbreak in 20 years but figures pertaining to outbreaks are not available. Viruses found in these countries include A, O and C types, and their variants. The European Commission for the control of FMD, which is sponsored by FAO of U.N., has published its fourth report that gives definite indication of active international cooperative efforts in the diagnosis, notification, and control of the disease.

RESEARCH DEVELOPMENTS

Tissue culture-V.E. virus systems have been developed to the point of practical utilization by several laboratories for serum-neutralization tests. These procedures afford a practical means of rapid diagnosis and typing of V.E. viruses.

The continuing, important cooperative field and laboratory studies of VS by the University of Wisconsin, the Agricultural Research Service of the United States Department of Agriculture and various state agencies is developing increasing knowledge regarding virus reservoirs, modes of transmission, delineation of enzootic areas and other information that is sorely needed for eventual solution of the complex VS problem. A progress report on these studies has been presented at this meeting. Continuation of this work is encouraged by your Committee.

Studies of immunization against VS with inactivated embryo-propagated virus also have been reported at this meeting by the Animal Disease and Parasite Research Division at Beltsville. Final determination of practicability of use of such products for the prevention of the disease will depend upon additional study.

Studies of FMD at Plum Island have revealed that the virus may be inactivated in varying extent by progressively increased or decreased pH adjustments or by various degrees of heat for different periods. It has been found, however, that, as might be expected, certain border-line treatments leave residual potentially infective virus. These studies are being continued, particularly in relation to characterization of the residual virus. By means of precipitation procedures and other treatment, including ultra centrifugation, workers at the Plum Island Laboratory have succeeded in processing a sufficiently pure preparation to demonstrate FMD virus particles with the electron microscope. The particles have been shown to be extremely small (approximately 21 mu in diameter) spheres which may be clumped by the addition of type-specific antiserum.

Also at Plum Island, studies have been made of the virucidal action of gaseous ethylene oxide against FMD virus. It has been found that under certain conditions, suspensions of highly virulent bovine epithelial virus may be inactivated to the point of non-infectivity for susceptible cattle severely exposed by inoculation. These studies are being continued in order to determine the effect of the gas upon different preparations under varying conditions.
In consideration of the long continuing absence of FMD in the United States of America, the current increasingly favorable VE situation, both compromised and complicated by the persistence of the clinically similar VS, your Committee strongly urges unrelaxed vigilance lest we be caught unaware and unprepared by an outbreak of serious consequences to the livestock industry and the nation.

**DISCUSSION**

**PRESIDENT GOOD:** Is there any discussion of the papers presented so far this morning?

**DOCTOR KUTTLER:** A breeder inquired of me recently about getting cattle out of Switzerland. I told him there was no chance because of foot-and-mouth disease. However, I suggested the possibility of using semen.

With this new development at Plum Island for identifying foot-and-mouth disease, I wonder if there is any possibility of checking semen to see whether or not it is safe.

**DOCTOR MAURICE S. SHAHAN [Long Island, New York]:** Doctor Kuttler's question is one that frequently arises in the minds of livestock breeders.

On the basis of our present knowledge, we regard semen as an equally dangerous method of introducing foot-and-mouth disease, equally dangerous with the importation of the animals themselves.

We would not regard (at this time, at least) utilization of electron microscope techniques for examination of semen as in any way adequate for the determination of the safety of such semen. In fact, we do not know how at this moment we could, with assurance, test semen and say it is safe.

So, for the time being at least, I believe the position of the Agricultural Research Service, at least, will be that there is not a practical means of importing semen from infected countries.

That is my effort, Doctor Kuttler. I don't know whether it answers your question or not.
REPORT OF THE COMMITTEE ON NOMINATIONS

Next is the report of the Committee on Nominations. Dr. Ralph West, Chairman.

Doctor West: Mr. President and Members of the United States Livestock Sanitary Association:

As you know, several years ago our Constitution was amended so that the Nominating Committee is made up of one individual from each of the four recognized districts, and one from Canada. The names of the members of the Nominating Committee are published in the proceedings of last year's meeting.

Your Nominating Committee this year has corresponded regarding proposed nominations, and has met as a Committee, all members being present except Doctor Wells from Canada, who was unable to be present. However, we do have a letter from him.

The following persons are hereby placed in nomination for the following offices in this Association for the ensuing year:

President—Dr. John G. Milligan, State Veterinarian, Montgomery, Alabama.

First Vice-President—Mr. F. G. Buzzell, Livestock Commissioner, Augusta, Maine.

Second Vice-President—Dr. James R. Hay, Director of Agriculture, Columbus, Ohio.

Third Vice-President—Dr. A. P. Schneider, State Veterinarian, Boise, Idaho.

President Good: Thank you, Doctor West. Are there any nominations from the floor?

Secretary Hendershott: Mr. President, I move that nominations be closed and that the President cast the ballot of the Association in favor of the men nominated—Doctor Milligan as President, Mr. Buzzell as First Vice-President, Doctor Hay as Second Vice-President, and Doctor Schneider as Third Vice-President.

Doctor Kuttler: Second the motion.

[The motion was put to a vote and was carried unanimously.]

President Good: I am very pleased to cast the ballot for Doctor Milligan as President, Mr. Buzzell as First Vice-President, Doctor Hay as Second Vice-President, and Doctor Schneider as Third Vice-President. [Applause.]

Will these new officers come to the platform, please?

Doctor West: I think it would be fitting at this time to announce the re-election of our Secretary-Treasurer by the Executive Committee.
REPORT OF THE COMMITTEE ON NOMINATIONS

PRESIDENT GOOD: Doctor Hendershott has been re-elected Secretary-Treasurer. Is there anyone who is against it? I think that is all taken care of. [Applause.]

DOCTOR WEST: It certainly was taken care of, but I didn’t think it had been publicly announced, and I wanted to mention it at this time.

PRESIDENT GOOD: Thank you for announcing it at this time. [Laughter.]

Doctor Reimenschneider, will you carry Doctor Milligan to the altar? Doctor Palmer, will you escort Mr. Buzzell? Doctor Hay is not here. Is Doctor Schneider here? Evidently not.

Doctor Milligan, it gives me great pleasure to turn the reins of this organization over to you. I hope you have a very successful year. [Applause.]

Congratulations, Mr. Buzzell. [Applause.]

[Dr. J. G. Milligan assumed the Presidency.]

PRESIDENT MILLIGAN: I certainly want to thank the members of this Association. For a long time I have wondered just how a country boy would feel if such an honor were bestowed upon him, and I can assure you that now I know how he would feel. I am scared to death.

I have a little comfort in knowing that this organization has a wonderful record for assisting its Presidents in performing the duties that are his responsibility, and I am sure that I shall have the same cooperation that our Presidents have had in the past. I want to assure you that I will try to carry on as others have before me, and I only hope I can do half as well.

Thank you. [Applause.]

Now may I present our First Vice-President, Mr. F. G. Buzzel. [Applause.]

MR. F. G. BUZZELL: I, too, appreciate the honor that has been bestowed upon me, and I hope that we can continue to make the United States Livestock Sanitary Association a good working tool for the livestock industry of this country.

Furthermore, I want to congratulate Doctor Good and Doctor Hendershott on the meeting we are just closing.

Thank you. [Applause.]

PRESIDENT MILLIGAN: Is there any new business to come before this Association at this time? If not, the meeting is adjourned.

[The meeting adjourned sine die at 12:30 P. M.]
CONSTITUTION AND BY-LAWS
OF THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

1 ARTICLE I—NAME
2 The name of this Association shall be “The United States Livestock Sanitary Association.”

4 ARTICLE II—PURPOSE
5 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.

17 ARTICLE III—MEMBERSHIP
18 There shall be three kinds of members—Official and Individual and Non-Voting Junior.

20 OFFICIAL MEMBERSHIP
21 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.

26 INDIVIDUAL MEMBERSHIP
27 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.
CONSTITUTION AND BY-LAWS

31 JUNIOR NON-VOTING MEMBERSHIP
32 Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.

ARTICLE IV—MEETINGS
36 The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS
38 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
42 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 General 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.
49 The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.
51 All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.
53 The First Vice-President shall be ex-officio chairman of the Executive Committee.
55 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.
58 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
66 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 the president in the event of the absence, disability or resignation of the president, first vice-president and second vice-president. He shall assume the chairmanship 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.

5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association. He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.
ARTICLE VIII—AMENDMENTS

The constitution of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting and further provided that the amendment has received the approval of the Executive Committee.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary-Treasurer.
President's Address.
Reading of Papers,
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers.
Adjournment.

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 Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association.
The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

**ARTICLE IV—QUORUM**

25 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 non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association.

The dues for official memberships shall be fifty dollars ($50.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
ROSTER OF MEMBERS BY STATES

ALABAMA

Official member
John Milligan
Individual members
Franklin A. Clark
George W. Cooper
George Ingram
B. N. Lauderdale
A. A. Leibold
John Milligan
C. H. Poitevint
P. M. Stone
Julian B. Taylor
Wm. L. Wake

ARIZONA

Official member
W. M. Thompson
Individual members
John Jacobs Farms
W. T. Lightle
Donald Miller

ARKANSAS

Official member
David Isben
Individual members
E. H. Peterson
Joseph P. Scott
Russell W. Williams

CALIFORNIA

Official member
J. E. Stuart
Individual members
P. A. Asplund
Raymond A. Bankowski
W. H. Boynton
Brennan & Laskey
V. C. Bunker
Hugh S. Cameron
N. H. Casselberry
John Chapman
G. L. Dayman
C. A. Delliquadri
Robert S. Dickson
Mary K. Dunlap
Thomas B. Eville
James N. Fulmor
V. E. Greening
T. J. Hage
R. H. Haight
Philip Haims
Joe Hart
Werner Paul Heuschele
R. C. Hubbard
L. M. Hurt
Donald E. Jasper
Harald N. Johnson
Arthur Kelly
John W. Kendrick
John King
C. R. Knight
Blaine McGowan
Kenneth G. McKay
W. Ofenheim
John C. Pace
F. L. Polisier
R. L. Phillips
Guy A. Railsback
Russell D. Richards
C. E. Robinson
Rebello Robusto
A. S. Rosenwald
San Diego County
F. H. Saunders
Kermit Schaaf
O. W. Schalm
R. J. Schermerhorn
Harold J. Schmidt
Col. M. D. Schroeder
C. R. Schroeder
Frank S. Scott
E. F. Sheffield
Jacob Traum
R. W. Wichmann
Gail R. Wilmuth

LOS ANGELES COUNTY

Official member
Robert J. Schroeder
Individual members
W. H. House
R. W. Hurt
R. W. McIntyre
L. F. Meier
W. L. Rottman
Robert J. Schroeder
W. A. Young

COLORADO

Official member
B. Shambaugh

Individual members
Wayne A. Anderson
W. W. Brown
W. A. Clark, Jr.
Charles L. Davis
N. Frank
R. M. Gow
Major Huff
Bryce R. McCrory
H. E. Schaulis

CONNECTICUT

Official member
J. V. Smith

Individual members
John W. Beck
Frank Ferrigno
J. Hwang
Erwin Jungherr
Bernard Lipman
F. K. Wills

DELWARE

Official member
W. R. Teeter

Individual members
Hiram N. Lasher
W. R. Teeter
Howard J. White
Clarence A. Woodhouse

DISTRICT OF COLUMBIA

Official member
C. D. Van Houweling

Individual members
James Hourrigan
W. O. Kester
Gen. J. A. McCallam
J. J. Martin
Albert R. Miller
C. H. Pals
B. C. Pier

A. F. Ranney
E. E. Saulmon
H. W. Schoening
Lloyd A. Spindler
A. L. Tellejohn
J. E. Williams

FLORIDA

Official member
C. L. Campbell

Individual members
Acree & Acree
I. P. Coulter
Jas. G. Fish
Jas. G. Fish, Jr.
J. H. Graves
Wm. F. Jackson
V. C. Johnson
W. R. Pritchard
Charles Reid
Wm. L. Sippel
Leonard E. Swanson
Carl Zillman
M. R. Zinober

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J. W. Mann

Individual members
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Osgood M. Bateman
G. A. Harner
T. J. Jones
J. Lieberman
R. W. Menges
C. J. Mikel
Adrian M. Mills
L. A. Mosher
Jack Russell, Jr.
L. E. Starr
James H. Steele
I. T. Sutton
J. M. Sutton
Ernest S. Tierkel

HAWAII

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E. H. Willers

Individual members
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George W. Murphy
E. H. Willers
MEMBERSHIP ROSTER

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A. P. Schneider
Individual members
Kenneth R. Hoyt
A. P. Schneider
J. Wendell Stucki

ILLINOIS
Official member
A. K. Merriman
Individual members
W. A. Aitken
American Shorthorn Breeders Assn.
David E. Bartlett
Paul D. Beamer
C. E. Blye
L. E. Boley
A. E. Bott
Anthony R. Bott
W. S. Buchanan
C. D. Carpenter
A. J. Coale
J. W. Cunkelman
Homer C. Curtis
L. R. Davenport
Milton R. Dunk
P. L. Du Puy
L. A. Dykstra
Lester E. Fisher
David R. Ganey
John O. Gwin
J. G. Hardenbergh
V. A. Haring
M. J. Harvey
A. B. Hoerlein
Clarence B. Hostetler
Robert H. Hoyt
Earl F. Huffman
G. W. Jensen
R. C. Kamm
E. C. Khuen
Carl H. Koons
B. L. Lake
A. A. Legner
A. J. Legner
Norman D. Levine
Edward M. Lynn
Fred C. Mau
Willis B. McCannon
A. C. Merrick
A. K. Merriman
Charles B. Michels
Mid-West Order Buyers
A. G. Misener
Robert L. Morin
Carlton H. Myers
Gilbert Novotny
James F. Palmer
Thomas H. Phillips
J. D. Ray
Herman C. Rinehart
C. M. Rodgers
Wm. Schwab
O. W. Seher
Jos. G. Sheaffer
H. L. Sparks & Co.
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M. H. Watkins
Olin G. Wheaton
Ed. J. Wilson
Harold E. Wilson
George T. Woods

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Individual members
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The Belt Railroad & Stock Yards Co.
Daly's Berry
F. H. Brown
John F. Bullard
Conner Prairie Farm
Charles R. Cutler
John M. Droge
S. M. Friedley
Geo. W. Gillie
F. O. Gossett
L. M. Hutchings
R. Vic Johnston
Hilmer L. Jones
R. C. Klussendorf
Bernard La Salle
Paul Leondis
McMahan-McClead
Karl Mayer
Harold E. Moses
James W. Newberne
C. E. Phillips
Virgil B. Robinson
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E. E. Slatter  
T. L. Steenerson  
Charles B. Swenson  
R. H. Waltz  
E. A. White  
Chas. J. York

IOWA

Official member  
A. L. Sundberg

Individual members  
Paul C. Bennett  
H. E. Biester  
Grant E. Blake  
I. H. Borts  
F. W. Bredahl  
D. A. Buchanan  
R. C. Bunge  
J. H. Clements  
George T. Edds  
B. J. Gray  
J. F. Harr  
Stanley L. Hendricks  
W. A. Higgins  
M. S. Hofstad  
R. L. Hounes  
E. D. Hubbard  
T. B. Huff  
Wilbur H. Hurst  
Norman K. Jungk  
Arden H. Killinger  
H. M. Kirk  
Jos. H. Krichel  
V. D. Ladwig  
C. D. Lee  
W. A. Liebsch  
Paul J. McAndrew  
H. L. McRillis  
I. A. Merchant  
P. C. Molgard  
Benjamin A. Moore  
Thomas W. Munce  
Grant B. Munger  
H. Stanley Nicol  
Wm. H. Olson  
Kenneth L. Ritchie  
John G. Salsbury  
Joseph E. Salsbury  
H. P. Sandberg  
E. Schneckloth  
Vernon Schneider

KANSAS

Official member  
A. G. Pickett

Individual members  
Armour & Company  
Ernest L. Boley  
L. M. Curts  
R. R. Dykstra  
Vernon D. Foltz  
E. J. Frick  
N. D. Harwood  
A. Kushner  
E. E. Leasure  
R. B. Meeks  
E. L. Mundell  
A. G. Pickett  
James A. Porter  
G. O. Schubert  
Louis H. Smith  
Marvin J. Twiehaus

KENTUCKY

Official member  
R. H. Singer

Individual members  
V. D. Bohannon  
Ben. J. Butler  
E. R. Doll  
Charles E. Eastin  
R. T. Gross  
John Healy  
F. E. Hull  
T. J. Stearns

LOUISIANA

Official member  
F. B. Wheeler

Individual members  
Herbert B. Elliott  
Lon E. Foote  
W. T. Ogleaby  
Albin G. Pass  
Frank B. Wheeler
### MEMBERSHIP ROSTER

**MAINE**

- **Official member**
  - F. G. Buzzell
- **Individual members**
  - Francis G. Buzzell
  - Stanford D. Merrill
  - J. F. Witter

**MARYLAND**

- **Official member**
  - A. L. Brueckner
- **Individual members**
  - John S. Andrews
  - Howard C. Barker
  - Paul Brandly
  - A. L. Brueckner
  - J. H. Collins
  - Cornelia Cotton
  - Chester N. Dale
  - Charles Durbin
  - Frank D. Enzie
  - W. S. Gochenour
  - Robt. T. Habermann
  - J. Walter Hastings, Sr.
  - Louis C. Heemstra
  - Raymond J. Helvig
  - W. R. Hinshaw
  - Allie A. Holbrook
  - David E. Hughes
  - Howard W. Johnson
  - A. M. Lee
  - John C. Lotze
  - Leroy Manlove
  - Chester A. Manthei
  - C. K. Mingle
  - L. O. Mott
  - I. M. Moulthrop
  - Oswald L. Osteen
  - William C. Patterson
  - Arthur H. Peck
  - L. J. Poelma
  - T. O. Roby
  - Martin H. Roepke
  - Harold E. Schaden
  - Wm. T. Shalkop
  - Claude A. Smith
  - Clarence H. Thompson, Jr.
  - R. E. Willie

**MASSACHUSETTS**

- **Official member**
  - E. M. Dwyer
- **Individual members**
  - Winthrop E. Brielman
  - E. R. Coon
  - Henry L. Foster
  - Donald Peck
  - Robert Tashjian
  - Henry Van Roekel

**MICHIGAN**

- **Official member**
  - L. Davisson
- **Individual members**
  - Ralph D. Barner
  - H. S. Bryan
  - Robert G. Carlson
  - C. H. Cunningham
  - Lee Davisson
  - F. E. Eads
  - Peter Gallitunas
  - Joseph H. Gainer
  - Hamilton Farm Bureau Coop. Inc.
  - E. V. Morse
  - Glen W. Reed
  - A. S. Schlingman
  - Oscar J. Sorenson, Jr.
  - H. J. Stafsseth
  - Gordon G. Stocking
  - E. J. Van Tilborg
  - Asa. Winter

**MINNESOTA**

- **Official member**
  - R. L. West
- **Individual members**
  - A. M. Anderson
  - Roger A. Ball
  - Beebe Laboratories
  - Elmer H. Braunworth
  - Homer C. Butler
  - John N. Campbell
  - Fred C. Driver
  - R. Fenstermacher
  - James J. Finson
  - Jas. A. Fitch
  - E. H. Gloss
  - Henry J. Griffiths
  - Don B. Hicks
MEMBERSHIP ROSTER

Harvey Hoyt
Jensen & Lundgren
H. H. Kanning
Howard C. H. Kernkamp
F. D. Knippling
C. A. Knorth
E. J. Kohler
David F. Long
George W. Mather
B. S. Pomeroy
Lee T. Railsback
Robert O. Rydell
Francis A. Spurrell
Frank C. Stiles
W. T. S. Thorp
R. W. Urbatsch
E. E. Wedman
James E. Wentworth
Dean W. Werring
R. Leland West
Ralph L. West
Paul Zollman

MISSISSIPPI

Official member
V. D. Chadwick

Individual members
Vernon D. Chadwick
B. T. Simms, Jr.

MISSOURI

Official member
L. A. Rosner

Individual members
Allied Laboratories, Inc.
American Hereford Association
Anchor Serum Co.
Robert L. Anderes
C. Herman Beckman
Robert K. Benn
F. W. Binkley
R. W. Boone
Elmer F. Brockman
John William Brown
E. A. Cahill, Jr.
Emmett L. Cary
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