Proceedings

SIXTY-THIRD

ANNUAL MEETING

UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

of the

UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

SHERATON-PALACE HOTEL
San Francisco, California
December 15, 16, 17, 18, 1959
Proceedings
SIXTY-THIRD
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

SHERATON-PALACE HOTEL
San Francisco, California
December 15, 16, 17, 18, 1959
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R. A. Hendershott, Trenton, New Jersey
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<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tr>
<td>Sept. 27–28, 1897†</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Texas</td>
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<tr>
<td>Oct. 11–12, 1898</td>
<td>Omaha, Nebraska</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kansas</td>
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<tr>
<td>Sept. 8–9, 1901</td>
<td>Buffalo, New York</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
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<tr>
<td>Sept. 23–24, 1902</td>
<td>Wichita, Kansas</td>
<td>*Mr. W. H. Dunn, Tennessee</td>
<td>*Mr. Wm. P. Smith, Monticello, Illinois</td>
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<tr>
<td>Aug. 23–24, 1904</td>
<td>St. Louis, Mo.</td>
<td>*Dr. J. C. Norton, Arizona</td>
<td>*Mr. Wm. P. Smith, Monticello, Illinois</td>
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<tr>
<td>Sept. 15–16, 1905</td>
<td>Guthrie, Okla.</td>
<td>*Mr. M. M. Hankins, Quanah, Texas</td>
<td>*Dr. S. H. Ward, St. Paul, Minn.</td>
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<tr>
<td>Nov. 28–29–30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
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<tr>
<td>Dec. 6–7–8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Montana</td>
<td>*Dr. O. E. Dyson, Kansas City, Mo.</td>
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<tr>
<td>Dec. 1–2–3, 1926</td>
<td>Chicago, Ill.</td>
<td>*Dr. L. Van Es, Lincoln, Nebraska</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<td>Nov. 30–Dec. 1–2, 1927</td>
<td>Chicago, Ill.</td>
<td>*Dr. C. A. Cary, Auburn, Alabama</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<td>Dec. 4–5–6, 1929</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<td>34</td>
<td>Dec. 3-4-5, 1930</td>
<td>Chicago, Ill.</td>
<td>Dr. A. E. Wight, Washington, D. C.</td>
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<td>35</td>
<td>Dec. 2-3-4, 1931</td>
<td>Chicago, Ill.</td>
<td>Dr. J. W. Connaway, Columbia, Mo.</td>
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<td>36</td>
<td>Nov. 30 - Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter Malcolm, Des Moines, Iowa</td>
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<tr>
<td>37</td>
<td>Dec. 6-7-8, 1933</td>
<td>Chicago, Ill.</td>
<td>Dr. E. T. Faulder, Albany, N. Y.</td>
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<td>38</td>
<td>Dec. 5-6-7, 1934</td>
<td>Chicago, Ill.</td>
<td>Dr. T. E. Robinson, Providence, R. I.</td>
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<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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<td>40</td>
<td>Dec. 2-3-4, 1936</td>
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<td>Dr. Walter Wisnicky, Madison, Wis.</td>
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<td>42</td>
<td>Nov. 30 - Dec. 1-2, 1938</td>
<td>Chicago, Ill.</td>
<td>Dr. D. E. Westmorland, Frankfort, Ky.</td>
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<td>43</td>
<td>Dec. 3-4-5, 1939</td>
<td>Chicago, Ill.</td>
<td>Dr. J. L. Axby, Indianapolis, Ind.</td>
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<td>44</td>
<td>Dec. 4-5-6, 1940</td>
<td>Chicago, Ill.</td>
<td>Dr. H. D. Port, Cheyenne, Wyoming</td>
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<td>45</td>
<td>Dec. 3-4-5, 1941</td>
<td>Chicago, Ill.</td>
<td>Dr. E. A. Crossman, Boston, Mass.</td>
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<tr>
<td>46</td>
<td>Dec. 2-3-4, 1942</td>
<td>Chicago, Ill.</td>
<td>Dr. I. S. McAdory, Auburn, Alabama</td>
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<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>
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<td>48</td>
<td>Dec. 4-5-6, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
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<td>49</td>
<td>Dec. 5-6-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
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<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>
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<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>
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<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>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyoming</td>
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<tr>
<td>62</td>
<td>Nov. 4-5-6-7, 1958</td>
<td>Miami Beach, Florida</td>
<td>Dr. John G. Milligan, Montgomery, Alabama</td>
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<tr>
<td>63</td>
<td>Dec. 15-16-17-18, 1959</td>
<td>San Francisco, Cal.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
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* Deceased.
† This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
‡ Reprinted in 54th Annual Report.
ADDRESS OF WELCOME

HON. W. C. JACOBSEN, Director of Agriculture
Sacramento, California

Mr. Chairman, Mr. Secretary and Ladies and Gentlemen and Guests of the United States Livestock Sanitary Association, I am very happy to see you. I see this is the Sixty-third Annual Meeting that is a long string of them and this is the first time you have met on the Pacific Coast. Welcome to the Pacific Coast, welcome to California and welcome to San Francisco.

I am particularly honored for this opportunity to say a few words to you because I was born in this town of San Francisco. My father was in the hay, grain and feed business and he had a penchant for developing pairs of light carriage horses and he used them for light draft too to provide exercise. And once in a while the horses would get sick and the local veterinarian was called to treat them. So I was exposed to members of the veterinary profession very early in life and later on in life I have been exposed to gentlemen of your profession and it has been a wonderful experience and a very helpful one.

Now you find us with what to your mind is very fine weather. It is, however, terrifically unusual for this time of the year. You would expect Californians to say something like this, but we are actually in critical need of moisture. The latest cooperative crop report shows that range and feed conditions are at 57 percent of normal, and throughout the state and that’s the lowest I have seen in a good many years.

I am delighted to see so many of my friends and I am amazed at the terrific scope of your program. The top flight and active committees that have just reported. The Advisory Committee to Agricultural Research Service I think is doing a terrific job and the Legislative Committee has also been active. If you combine those two dynamic committees they will be dynamic and that is not going to be any disadvantage to you.

Now while I have you here I want to tell you a little about California agriculture and some other matters that may be of interest to you. I would not want you to leave the state without knowing a little something about it. I am not one to brag so what I do say is said in all modesty. I want you to understand that.

California is generally noted for its fruits and vegetables. Well, that is true, but I think you may be surprised to know that our Animal Agriculture has gone way up. Our total annual cash receipts from farm marketings in 1958 was $2,852,769.00. Now, 48.2 percent of that was income from livestock and poultry and their products or $1,880,000.00. Now if you add the forage, the hay, the feed that is necessary to produce to maintain those animals and I am not saying anything about the food material we bring in, that would add up to 25 percent of the total that directly attributable to
ADDRESS OF WELCOME

livestock. That goes to show you how important your profession is to the agriculture economy of California.

The top six in 1958 of commodities that exceeded one hundred million dollars in value, cattle and calves first with 405,000,000, milk and dairy products, 338 million, cotton with approximately 300 million, chicken eggs, hay, grapes and tomatoes each had a value of over one hundred million dollars.

The livestock industry in California is the oldest in terms of our agriculture. The Spaniards brought livestock from Mexico. They also brought types of plants and seeds that formed the basis for crop production.

The Indians who were here were not very good farmers. They just cut and harvested what they could see. They did not do anything in production. So our agriculture production begins with the Spaniards and the Missions.

In the beginning livestock was produced for the pelts and wool. Cattle would bring about five dollars a head. There have been spectacular changes since that time of the gold rush days. Our crops are of the highly specialized character. Specialty crops if you please.

The University says we have 250 plus crops produced commercially. Because of the variety of soil and climate we can produce a wide variety of crops. So it is important that our agriculture research and education and regulatory services be maintained on a firm foundation.

We were discussing some of our possibilities last evening and it was said that with our climate and soil that if we could control our water supply there would be no limit to our production.

You notice I said that water was the limiting factor. We have plans underway to correct this but our progress in this direction is necessarily slow. After you talk for a while your mouth gets dry and if this was milk it would be a lot better.

Now I want to talk to you a little about our regulatory processes here. We have had our leaders in this state that had a lot of foresight. It comes to me because of my plant industry training.

It is my thought that if we are going to have a land agriculture a crop agriculture that we could not afford to be bitten by the pests that exist in other parts of the United States and other parts of the world. So we developed a program of plant quarantine to keep out diseases and pests if we possibly can have a system of detection or a survey that would provide that conditions could be detected in their incipient stage and eradicated.

Those are some fundamental precepts that I have been exposed to over the years—

Our animal people operate the same way and it was not difficult when our separate agencies were fused into one department to go along on that line of thinking.

I want to tell you about a quarantine conference they had in Washington on October 22nd that is just a short time ago in which the values of plant and animal quarantines enforcement integrated with the same general theories and precepts went a long ways towards showing the importance observing fundamental precepts I just talked about. It was regrettable that all of the people in our kind of work, all of those engaged in professional kinds of
agriculture dealing with plant and animal pests and diseases could not have sat in on that and heard. It was a remarkable presentation of the importance of agricultural quarantines.

One of the things that was very important was the recognition that the St. Lawrence Seaway now gives us 2,000 more miles of coastline. And the importance of extension of cooperation between the states and the federal government and the importance of cooperation between our Government and Canada. It was truly remarkable and I hope that sometime they see fit to develop that cooperation so it can be transferred to other areas in the United States so we can get the benefit of it.

Doctor Hay mentioned the recommendation in his report of the additional animal inspection stations like the one they have at Clifton, New Jersey. I assume that is what he referred to. We need such a station here on the Pacific Coast very badly.

Next to the quarantine enforcement and the eradication programs the one pest that is outstanding and touched upon extensively here by the two previous speakers is the brucellosis program. I will not go into any detail on that except to say that I hope that Congress will support the suggestions that were made by our National Association of State Departments of Agriculture and try to keep that hiatus that is developing extensively due to lack of funds will help narrow that down so we can get some emergency funds as early in 1960 as possible.

Well I don't want to keep you here too long. It should not take this long to say you are welcome, now should it.

I do want to make just one or two more comments.

Another precept that we were exposed to by our agricultural leaders was the importance of all of the agencies having to do with agriculture cooperate and coordinate their efforts.

Now one of the fundamentals of your organization was to do just that. That is one of the objectives and purposes of your meeting. So I am just bringing coals to Newcastle to talk about it. But it has been instilled in us. We have close cooperation here between the state and federal government and also with the local government. I can't stop without saying something about how remarkably proud we are of the research work that is being done in this state and I want to say that that goes for the animal field just as strongly and just as greatly as it is for the plant program with which I have a greater familiarity.

Now I said hello, I am very happy you are here and that you will enjoy California while you are here look around and have a good time, but for the love of Pete, if it rains don't crab about it for we certainly need the water. Thank you.
RESPONSE TO WORD OF WELCOME

J. G. MILLIGAN

Montgomery, Alabama

Director Jacobsen, those of us here from outside of California want to thank you for being so modest when you have so much to brag about. We also want to thank you for enlightening us about California agriculture. The story that you tell differs a little bit from that told by Richard Boone and other TV celebrities and when we came here we thought we’d find all the natives down by the river panning gold. But we find that you’ve got about 80 days without rainfall and all the streams are dry and they’re up here in town. I don’t think there’s any need for us to tell you how proud we are to be here in California with you. Quite a few of us traveled several thousands of miles through pretty rough country to get here and I’m sure there’s no need for us to tell you or to thank you for asking us here. We felt you ought to have meant it when you invited us. And we were proud to accept. I’m only sorry that we came at this time of year. I’ve been privileged to visit California in the summer several times in the past and things that I have seen on each visit made me a little bit envious of the people of this great state. I think that your mountains and valleys are unsurpassed when it comes to scenery. I have had the opportunity to see your vast fruit groves. I’m sure that the group here from Florida will have to admit that possibly you have them bested to some extent. I was privileged to visit your farmers market last Saturday and the array of vegetables displayed there certainly surpassed anything I ever saw before in my life. In times past I have had an opportunity to see some of your irrigated cotton lands. Those of us from the cotton belt who think that a bale to a bale and a half an acre is pretty good cotton can certainly take a lesson from you who are making three to five bales to the acre. I have seen some of your vast feed lots and huge dairies. We from other sections of the country can well take note of what you have done here. We only hope that you will allow us to continue to supply these feed lots and dairies with cattle. It certainly furnishes us with a great market. We also would like to thank you for inviting us to come out here once a year to play football in the Rose Bowl. And to show you just how much we do appreciate it one day we’re going to let you win a game. Again we want to thank you for allowing us this opportunity to come here and hope that we will be welcome to come back sometime in the future.

Thank you.
PRESIDENT’S ADDRESS

FRANCIS G. BUZZELL

Augusta, Maine

Members of the United States Livestock Sanitary Association, Ladies, Gentlemen, and Distinguished Guests: It gives me great pleasure to welcome you to the 63rd annual meeting of our Association. I sincerely hope that your attendance here will be worthwhile to you, as I know it will be to the Association.

This is the first time that this Association has held its meeting on the west coast, but to many eastern members it does not carry that remoteness that you might expect. Most of the older members remember family stories of grandparents whose brothers and uncles came into this area over a century ago to dig for gold in your mountains, and load hides on their ships for the eastern seaboard. I notice in the local telephone directory many names common to New England, and other eastern states, which remind us of those people who went around the Horn in their sailing vessels, as well as of those who traveled the overland trails, and encountered obstacles that were nearly insurmountable.

As we open this meeting, I believe that we should remind ourselves that our major function is the advancement of the livestock and poultry industry, and the protection of this industry from disease. The livestock and poultry industry accounts for a large percentage of our national agricultural income, as well as greatly contributing to a well-fed nation. The only way this advancement can take place is through a united front, all the way from the research man to the consumer. We, as a group, are mostly interested in production, and especially in the field of livestock health, which today is one of major importance. In fact, this Association was founded under the stress of livestock disease problems. It has functioned for 62 years, and without it, or a similar organization, our industry would not have made the progress it has. However, our progress need not stop here.

I am glad to welcome more livestock producers to our meetings, that you may become better acquainted with disease control problems nationwide, and that we may become better acquainted with your problems.

As I look back, in our small state, to the time when I was Chairman of the State Livestock Breeders Association, which included producers of dairy and beef cattle, swine and sheep, and we were trying to formulate a program for the control of brucellosis, little did we realize when we would become a certified state, or completely eradicate the disease. Eradication at that time seemed farther away than a maiden’s dream, but complete eradication should be realized soon in our state. It was evident to us then that we would have to get on an area basis, as we had made a dismal failure on a voluntary program. It was also evident that we could not indefinitely stand the heavy toll that the disease was taking from the industry.
Being a livestock breeder, and situated in the middle of New England, I have often thought that I was getting the dirty end of the stick when it came to exports. The import requirements were either laws or regulations, supported by the industry in the states where I was selling cattle, and regardless of laws or regulations, I had to meet the wishes of my customer, not only in breeding guarantees, but in health requirements. From time to time during the past few years, I have heard reference made to the fact that the chief livestock regulatory official is to blame for this or that in interstate health requirements. Since I have become associated with regulatory work, I have often wondered how livestock sanitary officials can obtain such power; how they can continue to force upon their own industry such requirements if they don’t have the backing of the industry in their own state, and I realize more and more that the importer usually writes the ticket. However, I believe that there has been more progress made this past year toward relaxing and adopting uniform interstate requirements than I have ever seen in any year before. I am beginning to think that I may live long enough to see uniform interstate requirements at least between certain areas of the country, and perhaps nationwide uniformity between the states. But again I want to emphasize the fact that state officials are only umpires, that the industry of the various states generally sets up the rules, either directly through laws, or indirectly by regulations arranged by sanitary boards or officials, after consideration of the wishes of the industry of their state, and if we believe in states’ rights, this system must remain. However, it does not mean that the livestock industry cannot eventually get its thinking unified on this subject on a national basis. Personally, I am encouraged by the progress that is apparently being made.

As many of you know, tuberculosis control and eradication has barely been holding its own during the past year or two in some areas, and remains a particular problem in at least a few states, which accounts for a large percentage of the nation’s infected herds. This problem has received special emphasis, particularly in some of those problem areas, and I hope that papers and testimony which will follow in this meeting will point out any weaknesses in our tuberculosis eradication program, that the industry may be better informed, and that steps may be taken to correct these conditions. I believe we should place more emphasis on eradication, and less on meeting minimum requirements for interstate movement, or retaining accredited status.

Generally speaking, the brucellosis program has progressed satisfactorily. More states and counties have been added to the certified group, and a few states are looking forward to complete eradication in the near future. This does not mean that we have no more problems. One of the problems in states with a very low incidence of disease is the vaccinated reactor (the only reactor or reactors in the herd), which appears in herds that may or may not be 100 percent vaccinated. The question confronting the disease control officials and the owner is: “Where did the infection come from, and is this animal a dangerous one in that herd, or in other herds?” In Maine and New Hampshire, where the herds are relatively free of brucellosis,
this vaccinated reactor is becoming more of a problem, and I hope that it will be possible to find the answer.

Apparently, federal funds have not been sufficient to carry out proposed brucellosis programs in some states as fast as such states were equipped to do the job. I understand that this situation has received the attention of the National Association of States Department of Agriculture, and our Association should correlate its efforts with that organization in trying to correct this situation. I would suggest that the Committee on Brucellosis, and the Advisory Committee to Agricultural Research Service, along with the Legislative Committee, bear this situation in mind, and give it careful and complete consideration.

Right here I would like to recommend that the Advisory Committee to Agricultural Research Service be made up of the three vice-presidents, the secretary, and a fifth member to be nominated from the area not represented by the vice-presidents, so that all four areas would be represented on this committee. If this recommendation were followed, committee members would be better qualified, as they would have had an opportunity to serve previous to their acting as chairman of this important Committee.

Hog cholera still remains the number one problem in swine diseases, and over the past several years this Association has made recommendations, some of which have been more or less adopted. However, the industry itself has made little progress in laying plans to eradicate this costly disease.

Vesicular exanthema has been declared eradicated. The cooking of garbage has helped not only in eradicating vesicular exanthema, but also helped control other diseases, and this practice should be continued.

As we control or suppress some diseases, others become of more importance. Among these could be classified leptospirosis, anaplasmosis, and several others, on many of which we need additional research work.

The opening of the St. Lawrence Seaway, and the increased use of airplanes, continue to add to our problems of keeping out foreign diseases, and increase the responsibilities of the Inspection and Quarantine Branch. I hope that we will not have to have a major disease outbreak before this situation is corrected.

Integrated types of production offer opportunities for real constructive and sound disease control programs. It also can become a hotbed of disease conditions in the case of the poultry, and the same may be true of integrated livestock operations. Many operators have not obtained qualified personnel, nor have they adopted sound disease control programs. In poultry integrated operations, many have depended upon vaccines and antibiotics instead of sanitation, and common sense disease control. In many areas, these integrated operations are carried on in the same locality with other producers, which has resulted in making it necessary that the latter follow the program adopted by the integrated producers, regardless of whether or not it is adapted to their operations. Recently, at the request of the poultry producers, research representatives, and the regulatory officials of the New England States have met to try to establish uniform measures for the control and eradication of certain poultry diseases in the New England
area. At that meeting, it was brought out that the indiscriminate use of vaccines in some areas was one of the biggest factors in disease control. This condition does not necessarily exist in the poultry industry alone, and is a condition which needs considerable thought. It appears that this is another case where state officials need the backing of the industry, that they may help protect the industry itself from some of the practices which have become common in certain sections, and which have been detrimental to the industry as a whole. Apparently, many states lack the authority and support to regulate the sale and use of certain biologics within their states.

Regardless of where your interest lies, whether it is in meat, milk, or other animals or animal products, or whether you are a producer, processor, manufacturer of biologics, or are interested in the field of marketing, or research, or are a veterinarian practicing in the field, or a state or federal official, the primary aim of each one is the more economical production of a better product for a more discriminating consumer through disease control.

In closing, I would like to thank the members of the Association for the honor that you have conferred on me. I would also like to thank the various officers and committee members who have worked, many of them throughout the year, on the various committees. I would especially like to thank Doctor Hendershott for his efforts during the past year, and I hope that the program for this meeting will meet with your approval.

I sincerely hope that everyone present will take part in the Committee hearings and in the discussion of papers so far as time is available. I especially want to thank Doctor Stuart and his staff for their efforts, and the planning that they have done to make this meeting a success.
PRESENTATION OF KEY TO PRESIDENT FRANCIS G. BUZZELL

DR. R. A. HENDERSHOTT

Trenton, New Jersey

Ladies and Gentlemen of the United States Livestock Sanitary Association:
As has been our custom in recent years, it is my distinct privilege and duty at this time to honor our President with a memento of our appreciation for his service as President of this Association during the past year.

In my experience over the last few years it has been my privilege to serve three outstanding members of the farm industry who are not professional men—that is they are not veterinarians—who have honored this Association by their service to us as President. I am confident and know I can state without fear of contradiction that you, Francis Buzzell, rate high amongst the presidents of this Association. You have given unselfishly of your time in the performance as President of this Association and have worked and stimulated activity on the part of your committees toward the advancement of the work and aims of the Association as a whole.

It is with a great deal of satisfaction that I am privileged now to present you with a token of the high esteem in which members of this Association hold you. In acknowledgment of your service we have had cast the key of the Association and had it incorporated in a tie holder.

It has been a decided pleasure for me to serve with you during the past year, and I feel I can speak for all of the members of the Association and particularly for the members of the various committees that we have enjoyed this year under your guidance and leadership. We salute you and congratulate you upon a most successful year.

Thank you, Ralph and members of the Association for this honor. It was a pleasant task for me to serve you and I trust I can conduct this meeting to your satisfaction. Thank you.
REPORT OF THE SECRETARY-TREASURER

R. A. HENDERSHOTT

Trenton, New Jersey

Mr. President, Members and Guests of the United States Livestock Sanitary Association: This year shall go into the records as one of the few in which our membership rolls topped one thousand. I am sorry that I cannot say topped ten thousand for in my opinion we should have at least that number interested and participating in the deliberations of the committees of this Association. We should have a large number of livestock producers as members and it should not be too great a task to materially increase our membership if each member would strive to interest one person each year to join. It is for this reason that along with your dues notice, copy of program and hotel reservation card, that an application of membership card is mailed you prior to the annual meeting.

It appears at this point that we are going to have better than average attendance at this our Sixty-third Annual Meeting. Many new faces are seen at the registration desk which opened yesterday morning.

This is the second year that we have set aside Tuesday for open meetings of the various committees. By open meetings I mean open to the public whether members or not, so that anyone interested with the work and action of the Committee may sit in and listen or request permission from the chairman to address the group on his ideas or express reaction to remarks made by other persons attending the committee hearing.

I think it is important that anyone connected with the livestock business understand that the control and eradication programs advanced for various livestock and poultry diseases have since 1897 had their origin in the committees of this Association. On our committees will be found the research men, the farmers and veterinarians private and governmental, both state and federal and extension service who are leaders in the disease condition assigned to the respective committees.

Yesterday it was very pleasing to me to observe the interest of livestock men who filled the large room assigned to the committee on brucellosis. These men were not there to just listen, they were speaking on the subject and presenting their views, their problems and offering suggestions as to how the brucellosis eradication program could be adjusted to their needs.

It is such interest as this that we admire and encourage. I counted 130 in the room and there was an overflow crowd of at least 40 persons outside the two double doors into the hall. A great many came to present their views which they did very concisely and objectively. I am certain their frank expression of what they thought should be done will be of great aid to the Committee.

I trust this activity will continue to grow. If we can stimulate the interest of our fine people the livestock owner, feeder, transporter and marketer and
SECRETARY-TREASURER

have them come in to these committee meetings and help to formulate the programs under which they are going to operate, then we shall have a healthy society of advantage to and certainly helpful and enjoyable to we who are legally charged to prevent, control and eradicate diseases of livestock. In connection with and emphasizing our desire for greater farmer participation in the work of this Association we shall at this meeting obtain executive committee approval of the proposed amendment to the constitution and by-laws which will provide for the election of two farmer representatives from each of the four districts, as set up by the United States Extension Service, to sit in and participate in the deliberation of the work of the Executive Committee of this Association. These farmer representatives will be chosen from amongst the active membership of the Association and shall be nominated and voted upon as are the officers of the Association.

During the past year we have witnessed the eradication of vesicular exanthema, a serious virus disease of swine, similar to foot-and-mouth disease and with its mode and rapidity of spread. Out of that experience came the almost universal program of the cooking of garbage fed to swine. The monthly inspection of garbage feeding farms to see that the garbage is being cooked and that the farms are being maintained in better sanitary condition than formerly; this feature is all to the good and will, if carried out religiously, prove to be a boon to the swine industry in the reduction of trichinosis, enteric infections and most important will prove to be of great aid in the eradication of hog cholera.

It is now a decade since I was instrumental in setting up a special committee on the Nationwide Eradication of Hog Cholera. The first meeting was convened at Purdue University at Lafayette, Indiana. The late Doctor Leslie M. Hutchings served as the chairman of the initial committee and the opening sentence of his report, which will be found on page 238 of the Fifty-fifth Annual Meeting report reads as follows:

“At the outset, your Committee wishes to emphasize it believes eradication of hog cholera is possible, but not so long as mass production and field use of virulent hog cholera virus is permitted.”

It might be well for us to reread the initial report of this Committee. It contains all of the facts needed to carry out an eradication program. Some of the things thought necessary a decade ago have been activated such as the almost universal cooking of garbage fed to swine. The legal exclusion of the use of fully virulent hog cholera virus by 32 states. More information on modified live virus vaccination and as well on dead culture immunization. Some things that we failed to obtain was the adoption of prompt reporting of outbreaks of hog cholera, the quarantine of farms on which natural outbreaks occur as well as those farms on which fully virulent virus is employed in the vaccination of swine. The cleaning and disinfection of vehicles used to transport animals from the farm. The construction of impervious, easily cleaned and disinfected pens at our markets. The refusal by markets to receive swine for sale without a certificate of health. The refusal of farm
people to harvest dollars out of the sale of exposed stock whenever disease strikes the stock and through this act further spread infection.

We shall have ready for distribution after the first of 1960 a pamphlet entitled “What One Should Know About Hog Cholera.”

What we need to get this program off the ground is a series of informative meetings at various parts of the nation sponsored by this Association in conjunction with Swine Breeders Associations along with the Animal Disease Eradication Division and the United States Department of Agriculture Extension Service.

These meetings could be patterned after those held regionally by the above agencies for the promotion of brucellosis eradication.

For years we have paid an unnecessary tax to hog cholera. It is past time when we should move to eradicate this disease. I trust I may live long enough to see it accomplished.

It has been a distinct pleasure for me to serve you as Secretary-Treasurer this year. As usual our President, Francis Buzzell, made some very good appointments on our committees and he and other officers of the Association have been very helpful. At this time I wish to express my appreciation of the work of our committees and also to those gentlemen who have given of their time and talent to prepare papers for presentation at this 63rd Annual Meeting. A glance at the program will give you an idea of the multiplicity of topics that we shall have the pleasure of listening to.

All of us can take pride in the accomplishments of this Association, let each one pledge himself to present the opportunity of membership to his fellowman then let us all move forward and eradicate the number one swine disease, hog cholera.

Thank you.
REPORT OF AUDITOR

Officers and Members,
United States Livestock Sanitary Association,
33 Oak Lane,
Trenton, N. J.

I have examined the Statement of Receipts and Expenditures of the United States Livestock Sanitary Association for the period 1958-1959, and the books and records relative thereto, including the checking and verification of all deposits, and the examination of the endorsements on all cancelled checks, for the period under audit.

In my opinion, the Statement of Receipts and Expenditures attached hereto, presents fairly the financial condition, and the results of the operations of the Association for the 1958-1959 period audited and examined by me.

ANDREW J. GROVE, Examiner,
Department of Banking and Insurance,
State of New Jersey.

December 11, 1959.
MEMORIAL SERVICE

M. N. RIEMENSCHNEIDER

Mr. President, Members of the Association, Ladies and Gentlemen: To the best information available to me the following members have passed away during the past year.

RAYMOND R. BIRCH

Dr. Raymond R. Birch (COR '12), 78, Ithaca, N. Y., widely known authority on hog cholera, bovine tuberculosis, brucellosis and for many years on the faculty of the New York State Veterinary College at Cornell University died on July 26, 1959.

Doctor Birch received a B.S. degree in Agriculture from Kansas State College in 1906 and then went to the Philippine Islands where he was an inspector with the Bureau of Agriculture and a specialist in animal industry for three years. While there, he became interested in veterinary medicine, after witnessing the critical need for animal disease control in the Islands. He then entered Cornell where he received his D.V.M. degree in 1912.

After graduation, he joined the veterinary faculty as superintendent of the veterinary experiment station and was engaged in hog cholera serum production for a time before engaging in brucellosis research, an interest that extended over the next 25 years. He rose to the position of professor of veterinary research and continued as superintendent of the experiment station until his retirement in 1939.

He was an International Education Board scholar and studied in Europe in 1926. Doctor Birch served on a number of professional and scientific bodies, notably as a consultant on the International Committee on Infectious Animal Diseases, Marshall Plan countries in 1951-52, and the World War II Committee on Procurement and Assignment.

In 1949, he received the Borden Award and Medal administered by the A.V.M.A., for his research on brucellosis and on the practical application of his findings in the control of bovine brucellosis.

WILLIAM H. BOYNTON

Dr. William H. Boynton (Cornell '08), passed away in Berkeley, California on November 10, 1959, at the age of 78. His life was devoted to research which was responsible for many advances in veterinary science. His contributions were particularly significant in the study of such diseases as rinderpest, anaplasmosis and hog cholera.

Doctor Boynton attended the University of California for his undergraduate work from 1903 to 1906 and then went to Cornell to study veterinary medicine. He received his Doctor of Veterinary Medicine degree in 1908 and stayed there for two years as an instructor in pathology. He then went
MEMORIAL SERVICE


A native of Columbus, Ohio, Doctor Collins served with the United States Department of Agriculture for seven years following graduation, then entered the Food and Drug Administration in 1942 as an assistant veterinarian. He carried on research at the FDA laboratories in Beltsville until assigned to the administrative headquarters in 1946. Doctor Collins was made veterinary medical director in 1953 and chief of the veterinary medical branch of the agency's Bureau of Medicine. In addition, he was the author of many scientific technical papers and developed a standard method, still employed, for testing the efficacy of poultry coccidiostats.

John I. Curtis (COL) passed away on October 10, 1959 at the age of 71. He was a graduate of Utah State University and Colorado State College, School of Veterinary Medicine. He practiced Veterinary Medicine for 18 years in Richfield, Utah, after which he became assistant to Dr. W. H. Hendricks. When the latter retired he became State Veterinarian.

Doctor Curtis retired as State Veterinarian in March 1956 and was employed in the Poultry Inspection work of the United States Department of Agriculture at the time of his death.

John L. George (KSC '32), 53, Chester, Nebraska, former Nebraska State Veterinarian died June 30, 1959.

Born in Norton, Kansas, Doctor George began his practice in Chester immediately after graduation from Kansas State and was still practicing.
there at the time of his death. He interrupted his practice there for only 18 months in 1934-35 to work with the United States Department of Agriculture in Oklahoma and Indiana.

In 1953, Doctor George was selected as State Veterinarian for Nebraska and served until ill health necessitated his resignation in 1958. He was an active member of both state and national organizations, serving on many committees and having made many contributions.

WILLIAM S. GOCHENOUR, SR.

Dr. William S. Gochenour, Sr. (UP '13), 67, once a resident of Indianapolis, Indiana, and former vice-president of Pitman-Moore Company, died on June 11, 1959. He had retired and moved to Florida.

In 1915, he joined the Bureau of Animal Industry field inspection force and was engaged in foot-and-mouth disease eradication work in 1914. He was later transferred to the Pathological Division in Washington. There, he was engaged in studies on shipping fever, anthrax, and other livestock diseases and their immunizing agents until 1937. During World War II, he was active in allied joint research on bacteriological warfare.

LESLIE M. HUTCHINGS

Dr. Leslie M. Hutchings (MSU '40), 43, former head of the Department of Veterinary Science at Purdue University and first dean of its newly established School of Veterinary Science and Medicine, outstanding veterinary pathologist, and internationally known authority on brucellosis, transmissible gastroenteritis, and other swine enteric diseases, passed away on July 22, 1959.

Born at Portland, Maine, September 13, 1915, Doctor Hutchings received his preliminary education in the local schools and then entered the University of Maine, at Orono, from which he received his B.S. degree in 1937. He was awarded the D.V.M. degree from Michigan State College (now University) in 1940 and an M.S. degree in 1942. He was an assistant in pathology from 1937 to 1940 and then served as research veterinarian until 1942 while studying for his M.S. degree.

In 1942, Doctor Hutchings accepted appointment as associate pathologist in the Department of Veterinary Science at Purdue. He was awarded a Ph.D. degree in Pathology and promoted to professor in 1947. He became head of the Department in 1950, succeeding the late Dr. C. H. Donham. In 1947, he won the Sigma Xi Research Award and also was named "Outstanding Young Man of Indiana" by that state's Junior Chamber of Commerce.

He was director of the Indiana State Animal Disease Diagnostic Laboratory for a number of years and was a member of the Indiana State Livestock Sanitary Board. He was an active member of both state and national organizations. Some of which were the National Research Council's committees on animal health, brucellosis, and leptospirosis; a member of the panel of experts on brucellosis of the World Health Organization, FAO, since 1950. He was also a member of the American Society of Bacteriologists, the Confer-
ence of Research Workers in Animal Diseases of North America, the International Congress on Tropical Diseases and Medicine, and other professional and scientific bodies, including the honorary scientific Society of Sigma Xi.

WILL J. MILLER

Will J. Miller, 74, Topeka, Kansas, passed away on March 10, 1959. Born at Osage City, Kansas, in 1884, Mr. Miller graduated from Kansas University in 1908. He was livestock sanitary commissioner from 1938 to 1949 and in 1942 was elected president of the National Assembly of Chief Livestock Sanitary Officials.

In 1944, he was proposed for and unanimously elected to honorary A.V.M.A. membership in recognition of his tireless and successful efforts in obtaining congressional approval of appropriations for the upward reclassification of federal veterinarians and increasing their salaries.

F. E. MOLLIN

F. E. Mollin, 71, passed away October 16, 1958. He was a native of Nebraska and has been associated with the livestock industry and ranching all during his active life. Mr. Mollin became Executive Secretary of the American National Cattlemen's Association, April 1, 1929, at which time he moved to Denver, Colorado. He was a tireless worker for the organization he represented. Mr. Mollin was instrumental in increasing the membership of the organization from a few thousand members in 12 affiliated state groups to 150,000 cattlemen in 29 states and 120 local organizations affiliated in the nation. Besides his work as secretary of the American National Cattlemen's Association, he was actively engaged in ranching operations in Nebraska, South Dakota and Colorado. Mr. Mollin gave freely of his time and effort to serve on many committees of various organizations. He was past president of the United States Livestock Sanitary Association.

RICHARD L. HOUMES

Dr. Richard L. Houmes (ISC '47), 34, Central City, Iowa, died March 26, 1959, after an illness of several weeks.

Doctor Houmes had practiced in Central City since graduating from ISC in 1947.

H. J. O'CONNELL

Dr. H. J. O'Connell (MCK '18), 67, Madison, Wisconsin, a veterinarian for the state and federal governments for more than 35 years, died in Madison on April 27, 1959. Doctor O'Connell joined the state's Department of Agriculture in 1924, and after many years as field veterinarian, was named chief of the department's animal disease division in February, 1951. Head of the tuberculosis eradication work practically since its inception, Doctor O'Connell was also in charge of the Division when a campaign against brucellosis was launched in 1951. At the time of Doctor O'Connell's
retirement in January, 1957, Wisconsin was the first major dairy state to be declared certified brucellosis-free. After reaching the state's mandatory retirement age, Doctor O'Connell continued to head the tuberculosis control work, under federal appointment. Also in 1957, Doctor O'Connell received an award from the United Stated Department of Agriculture's Agricultural Research Service for his work in Wisconsin's campaign against brucellosis.

I respectfully request all present to arise and remain standing in silent prayer for the peaceful repose of the souls of our deceased colleagues.

SILENT PRAYER

Thank you ladies and gentlemen for your respectful participation.

On this occasion your speaker feels very humble and inadequate to properly memorialize these splendid men. It would require a person of greater oratorial ability than I to begin to do justice to them, but certainly their accomplishments, deeds and virtues will endure much longer than all the words that may be said here today.

They have left us an inheritance wherein we are all debtors to them for many things we cherish. These men gave much in toil and sacrifices that we might share in the fruits of their labors. While this inheritance gave us privileges and makes advancements easier, it also imposes responsibilities which we must regard as sacred trusts for the future, never to be exploited or wasted, but to be enriched and passed on to future generations.

They have gone from us to higher realms of immortality. We will cherish their lives for their faithful service, their public and private virtues, and in our hearts build a monument, precious to their memory.

Thus let us memorialize them this day and resolve to carry on the work along the paths so well emulated by their lives.
REPORT OF THE ADVISORY COMMITTEE TO THE AGRICULTURAL
RESEARCH SERVICE, UNITED STATES DEPARTMENT
OF AGRICULTURE

J. R. Hay, Chairman, Chicago, Illinois; R. A. Hendershott, Trenton, New
Jersey; W. L. Bendix, Richmond, Virginia; A. P. Schneider, Boise,
Idaho, H. G. Geyer, Columbus, Ohio.

The established pattern of operation by the Advisory Committee to the
Agricultural Research Service was continued during 1959. Four meetings
were held, with two in Washington, one in Kansas City, and one in San
Francisco.

The first Washington meeting was held February 2-3, 1959, and was
devoted to a complete review of the Agricultural Research Service budget
for fiscal 1960. At this time, when advised by Agricultural Research Service
of the request for reduced brucellosis funds, the Committee strongly recom-
mended that the reduction in services required by the cut be in the field of
new work rather than reducing the rate of work already in progress.

The construction of four additional quarantine stations was recommended.
The Committee was advised that these would be included in the United
States Department of Agriculture five year building needs and urged before
Congress for the fiscal 1961 budget.

The Committee discussed in detail the procedure for testing all biologicals.
Two and one-half billion doses are produced each year with ninety-three
percent being live-products. The Committee took a strong stand that Agri-
cultural Research Service begin to develop procedures for the testing of all
biologicals. Testing facilities to be made available at the National Disease
Laboratories at Ames, Iowa.

The Committee advised Agricultural Research Service that they considered
the following items of great importance when the 1961 budget was discussed
at a later date: 1. $3,000,000 for operation of National Disease Labora-
tories, Ames, Iowa; 2. $1,000,000 for equipment and supplies for the
laboratory; and, 3. two and one-half to three millions to convert Beltsville
Laboratory to Parasite Research Center.

The second Washington meeting was held April 16-17, 1959 and was
devoted to meeting with division chiefs for a discussion of programs, proj-
ects, and future aims plus a meeting with budget committee of Agricultural
Research Service on the 1961 budget.

For the Animal Inspection and Quarantine Division, the Committee re-
iterated the same position taken each year since 1954, for the critical needs
for adequate facilities and personnel along the Canadian border. With the
opening of the Seaway, this program takes on additional importance. A
specific request was made for five new facilities along the Canadian border
to handle the marked increase in the importation of animals along this area.
The fencing of the Mexican border was discussed in detail and recommen-
dations for $100,000.00 was suggested for adding to and keeping in repair the present 200 miles of fence.

For the Meat Inspection Division, it was recommended that continuing consideration and investigation be given to the proposal that meat inspected under a proper, adequate, State program be allowed to move in interstate traffic. The Committee further recommended and endorsed adequate funds for the meat inspection service but felt that the cost of humane slaughter had not been sufficiently evaluated. The Committee was advised that a supplemental appropriation of $500,000.00 for 1959 was essential, that this amount of increase was in the 1960 budget and was adequate, and in 1961 when the effect of the humane slaughter would be felt, that no specific amount had been requested as the budget was presented to Congress.

For the Animal Disease Eradication Division, the Committee expressed concern over the dropping of five million dollars for brucellosis control and felt that by so doing it precluded ever accomplishing total certification or total eradication of brucellosis. Need for continuing efforts by all agencies, both state and federal, to overcome the prevalence of complacency in tuberculosis control was reiterated. The Division was requested to take the lead in unifying efforts toward total eradication of scabies. Such an effort to be coupled with more research into the use of new insecticides and the problem of residues.

For the Animal Disease and Parasitic Research Division the Committee made a specific recommendation for two million dollars be added to the 1961 budget to complete the equipping of the National Animal Disease Laboratory at Ames, Iowa. The budget committee of Agricultural Research Service was urged to accept a realistic and minimal four and one-half million dollars for the immediate research needs of the nation's livestock industry at Ames. The urgent need for adequate control over the manufacturing and licensing of animal and poultry biological products was considered and it was recommended the $500,000.00 be considered as minimal to start this work and to be in addition to and not including the Division's previous appropriation. The need for diagnostic service in the regulatory field served by this Division was discussed and the Committee recommended a minimal $600,000.00 be provided for this service and that facilities at Ames be made available for this purpose. The Committee further urged that the conversion of the Beltsville Animal Disease Station be a National Parasite Research Laboratory be given high priority.

The Chairman of the Advisory Committee to Agricultural Research Service wishes to take this opportunity to express his appreciation to the other members of the Committee and to the staff of Agricultural Research Service for the splendid cooperation given during this past year. Having served as chairman of the first Advisory Committee in 1954 when it was started in the National Assembly of Livestock Regulatory Officials it is easy to observe the improvement in understanding which has developed between the U. S. Livestock Sanitary Association and Agricultural Research Service during the last six years. There is, however, one area where the Advisory Committee believes that further improvement could be made. This was discussed at the
Kansas City meeting and would be to combine the Advisory and Legislative Committees of this Association. This would give better continuity of effort and with the structure of the Advisory Committee, it gives experience to the officers of this Association in the problems of budget, programs and legislation at a national level. I would further recommend that members of this Committee be designated as delegates and alternates to the National Association of State Department of Agriculture. Such a move would further broaden the experience of the officers, give guidance and continuity to resolutions considered by both Associations and provide a liaison of effort which seems to increase in importance each year.
REPORT OF REPRESENTATIVES TO THE ANNUAL MEETING OF ASSOCIATION OF STATE DEPARTMENTS OF AGRICULTURE


The 1959 annual meeting of the National Association of State Departments of Agriculture was held at the Andrew Jackson Hotel in Nashville, Tennessee, October 19-23, 1959. In attendance at this meeting from the United States Livestock Sanitary Association were Dr. C. E. Kord of Tennessee, Dr. J. G. Milligan of Alabama, and Dr. W. L. Bendix of Virginia. In addition, Dr. T. J. Grennan, the State Veterinarian of Rhode Island, and Dr. A. K. Merri- man, the State Veterinarian of Illinois, were present.

Doctor Milligan, as the immediate past president of this Association, gave an interesting and informative talk on the problems encountered in disease control and discussed fully some of the future disease problems with which this Association will be coping. Doctor Milligan's talk was mimeographed by the Tennessee Department of Agriculture and copies were distributed to all those in attendance.

There was much interest in the problems of livestock and poultry disease-control work on the part of the commissioners, and the delegates from this Association were invited to attend their committee sessions and to participate fully in their deliberations. As a result of their disease-control committee, the following resolutions were unanimously passed on the final day of the meeting:

RESOLUTION NO. XI—AGRICULTURAL QUARANTINE INCREASE

WHEREAS, the National Association of State Departments of Agriculture in 1958 passed resolution No. 20, reiterating an earlier request that Agricultural Research Service, United States Department of Agriculture, include not less than one million dollars of additional funds in its 1960 budget for use in strengthening agricultural quarantine (plant and animal) inspection at ports of entry; and

WHEREAS, no additional funds were provided for that purpose in the appropriation for fiscal year 1960; and

WHEREAS, the danger of the introduction of foreign pests and diseases of plants and animals has been made even more serious by the steadily growing volume of foreign traffic and commerce; and

WHEREAS, repeated recent interceptions of snails, khapra beetle, and golden nematode in general cargo such as automobiles, steel products, and canned goods, have demonstrated the need for more thorough inspection of non-agricultural cargo if such pests are to be prevented entry; and

WHEREAS, more long-range jet aircraft are overflying port and border airports and landing at interior airports, thus creating additional inspection problems; and

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WHEREAS, this growing volume of traffic is also forcing the opening of new sea and border ports of entry and causing the expansion of existing international airports, thus creating still additional avenues for pest entry;

Now, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that the Association recommends to the Congress of the United States that an increase of $1,500,000 be appropriated to the Agricultural Research Service, United States Department of Agriculture, for the strengthening of Agricultural Quarantine Inspection for the fiscal year 1961;

and

BE IT FURTHER RESOLVED, that a continuing study be conducted to determine the adequacy of funds for Agricultural Inspection to meet the ever-changing situation.

RESOLUTION NO. XXII—SENATE BILL 864, 86TH CONGRESS

WHEREAS, Senate Bill 864 of the 86th Congress, First Session, entitled “An act to provide greater protection against the introduction and dissemination of diseases of livestock and poultry, and for other purposes,” has passed the Senate of the United States of America in Congress assembled; and

WHEREAS, the said act is now in the House of Representatives of the United States of America in Congress assembled and has been referred to the Subcommittee on Livestock and Feed Grains of the committee on Agriculture of the House of Representatives and is now under consideration by said subcommittee; and

WHEREAS, the authority provided in this bill is essential for the United States Department of Agriculture to cope with emergency outbreaks of animal diseases and to discharge its responsibilities in the prevention and spread of such diseases; and

WHEREAS, the Association has supported this legislation as amended; and

WHEREAS, questions have been raised as to the possible conflict of this method of compensation provided in this bill and the laws of several states;

Now, THEREFORE, BE IT RESOLVED, by the 41st Annual Convention of the National Association of State Departments of Agriculture meeting in session in Nashville, Tennessee, October 19-23, 1959, that its Executive Committee work actively with and re-affirm to the House of Representatives of the United States Congress and the various members of the United States Senate its support of S. 864 and HR 7313 with amendment, if necessary to remove any possible conflict between this bill and the various state laws as in the payment of compensation paid to the owners.

RESOLUTION NO. XXIII—MASTITIS CONTROL

WHEREAS, a condition in dairy cattle known as mastitis is causing a heavy financial loss to dairy owners throughout the nation annually, and there is a marked division of opinion as to whether mastitis is a distinct disease or the result of several types of infection and contributing factors;
NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that it requests its Executive Committee to urge the United States Secretary of Agriculture to conduct an extensive coordinated research program on mastitis so the great loss from this disease be reduced.

RESOLUTION NO. XXIV—INTERNATIONAL BOUNDARY FENCE

WHEREAS, the livestock industry of the southwestern areas of the United States continues to suffer exposure of its domestic animals to diseases borne by drifting animals from foreign sources because of the disrepair or complete destruction or absence of the International Boundary Fence, which was intended to, in fact, exist as a protection against straying animals across the border strip between the Rio Grande River and the Pacific Ocean; and

WHEREAS, such lack of restraint upon the indiscriminate movement of infected animals from foreign sources through such a neglected fence in large measure nullifies the value of careful quarantine inspections and holding of animals at established border entry points to prevent introduction of injurious diseases; and

WHEREAS, during the past four years there have been numerous instances of hazardous animals having drifted across said unprotected border strip, resulting in additional expense in carrying on necessary protective measures to insure against disease outbreaks;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that its Executive Committee work toward the reintroduction of legislation comparable to S. 76 of the 84th Congress of the United States to make provision for the construction and/or repair of an adequate boundary fence, and the granting authority and finances to the Secretary of Agriculture for the adequate maintenance of such fence so that it will, in fact, serve as a necessary protection against indiscriminate entrance of diseased livestock.

RESOLUTION NO. XXV—HOG CHOLERA VIRUS

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

WHEREAS, hog cholera cannot be eradicated while virulent hog cholera virus is in general use; and

WHEREAS, many states have passed laws prohibiting the sale of virulent hog cholera virus, except for experimental purposes and then only by permit issued by the responsible state agency;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that its Executive Committee urge the United States Secretary of Agriculture to sponsor legislation which will prohibit the movement and sale between the various states of swine which have been immunized with virulent hog cholera virus.
RESOLUTION NO. XXVI—HOG CHOLERA

WHEREAS, there has been developed a method of immunizing swine against hog cholera known as modified live virus hog cholera vaccine; and

WHEREAS, it is not necessary to use virulent virus, which is a means of perpetuating the disease; and

WHEREAS, hog cholera cannot be eradicated while virulent hog cholera virus is in general use; and

WHEREAS, many states have passed laws prohibiting the sale of virulent hog cholera vaccine except for experimental purposes and then only by permit when used by the responsible state agency;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that its Executive Committee urge those states which have not passed legislation prohibiting the sale of virulent hog cholera vaccine to do so as soon as possible, and to also enact legislation requiring the use of modified live virus hog cholera vaccine as the method for immunizing swine against hog cholera.

RESOLUTION NO. XXVII—TUBERCULOSIS ERADICATION PROGRAM

WHEREAS, bovine tuberculosis poses a threat to the health and well-being of the nation’s approximately 175 million people; and

WHEREAS, considerable progress has been made toward the eradication of tuberculosis in the United States through the expenditure of more than 350 million dollars of federal and state money; and

WHEREAS, due to the extensive movement of livestock it is virtually impossible for any individual state or group of states to achieve complete eradication without a comprehensive eradication program being followed in all states; and

WHEREAS, because of higher costs, the net amount of testing for tuberculosis has become less and less each year, even though the appropriation for such has remained the same; and

WHEREAS, the disease control benefits, gained through the expenditure of tremendous sums of money over many years, may be lost as a result of suspension of certain phases of the program even though complete eradication of tuberculosis now seems feasible; and

WHEREAS, the number of cattle showing a sensitivity to the tuberculin test has materially increased during recent years, posing new problems in control and eradication of tuberculosis and necessitating funds in excess of existing appropriations;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that this Association request the United States Secretary of Agriculture to diligently and forcefully seek adequate appropriations from Congress to complete the tuberculosis eradication program in all states; and
BE IT FURTHER RESOLVED, that the United States Secretary of Agriculture in cooperation with each Commissioner, Secretary or Director of Agriculture re-evaluate the effectiveness of the tuberculosis eradication program and the expenditures therefore in his state; and

BE IT FURTHER RESOLVED, that each member of this Association be requested to send a copy of this resolution to each of his respective Senators and Congressmen along with a letter explaining the need for funds to complete the eradication program already under way in his state.

RESOLUTION NO. XXVIII—UNIFORM BACK TAGGING PROGRAM

WHEREAS, recently approved regulations pertaining to the brucellosis program permit the recertification of certain states through the devices of making use of a cull and dry cow testing program; and

WHEREAS, many states export large numbers of cull and dry cows to adjoining states for slaughter; and

WHEREAS, these cows lose their identity when they cross state lines and the exporting state loses credit toward its recertification program;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture, assembled in convention at Nashville, Tennessee, October 19-23, 1959, that the various State Departments of Agriculture be respectfully urged to hasten adoption of the uniform back tagging program which will make it possible to trace the identity of cattle in slaughterhouses in states other than the state of origin.

RESOLUTION NO. XXIX—SCRAPIE DISEASE QUARANTINE AND RESEARCH

WHEREAS, scrapie is a serious disease of sheep for which there is no known remedy except that of condemning the contagious and infectious sheep for slaughter; and

WHEREAS, there have been instances in which this disease has been introduced into the United States by importation of sheep from flocks and/or premises in foreign countries known to be infected with scrapie; and

WHEREAS, there have been instances in which this disease has been introduced into a state by importation of sheep from flocks and/or premises from another state known to be infected with scrapie; and

WHEREAS, the importation of such sheep into the United States from foreign countries and into states from the various other states has resulted in the United States Department of Agriculture and the various states being required to expend large sums of money for indemnity;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that its Executive Committee urge the United States Department of Agriculture to establish regulations which will prohibit the entry of sheep into the United States from foreign countries and which will prohibit movement of sheep from the various states, from flocks where scrapie is known to have existed or from premises whereon the disease has existed in the last five years immediate past; and
BE IT FURTHER RESOLVED, that intense research be undertaken by the Agricultural Research Service on this disease.

RESOLUTION NO. XXX—SCABIES

WHEREAS, cattle and sheep cannot be raised profitably when affected by scabies and the widespread incidence of the disease threatens the cattle and sheep industries; and

WHEREAS, many years of effort and tremendous sums of money have been expended in freeing the western range states of this scourge; and

WHEREAS, despite all possible efforts with the limited funds available in recent years, the program has reached a stalemate in many areas; and

WHEREAS, eradication is entirely feasible if state and federal programs are materially strengthened;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that additional state and federal funds be made available to accomplish eradication, and that the states and the United States Department of Agriculture be urged to secure the funds necessary to accomplish this end.

RESOLUTION NO. XXXI—BRUCELLOSIS ERADICATION PROGRAM

WHEREAS, considerable progress has been made toward eradication of brucellosis in many states; and

WHEREAS, vast sums of money both federal and state, have been expended to reduce the extent of this disease to a point that virtual eradication is now feasible; and

WHEREAS, due to extensive interstate movement of livestock it is practically impossible for any individual state or group of states to achieve complete eradication without comprehensive eradication programs being followed in all states; and

WHEREAS, a reduction of federal allotments available for such eradication program has resulted in the suspension of certain phases of the program in some states so that current programs are inadequate to assure control; and

WHEREAS, the disease control benefits gained through the expenditure of tremendous sums of money over many years may be lost as a result of suspensions of certain phases of the program; and

WHEREAS, brucellosis poses a threat to the health and well-being of the nation's 175 million people;

NOW, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that this Association request the United States Department of Agriculture to diligently and forcibly seek adequate appropriations from the Congress of the United States so the eradication program for brucellosis may be completed in all states in the shortest possible time; and

BE IT FURTHER RESOLVED that each member of this Association be requested to send a copy of this resolution to his respective Senators and Con-
gressmen along with a letter explaining the need for funds to complete the eradication program in his state.

RESOLUTION NO. XXXII—FUNDS FOR FEDERAL-STATE BRUCELLOSIS ERADICATION PROGRAM

WHEREAS, an accelerated brucellosis eradication program was encouraged by federal authorities and by Congressional action through allocation of $24,000,000 for such purpose in 1954, followed by comparable allocation in succeeding fiscal years and setting a tentative goal for completion by mid-1960; and

WHEREAS, many states in reliance thereon stepped up their programs commensurately, resulting in primary certification in 21 such states, although some states, through no fault of their own, could only adopt the program in slower stride; and

WHEREAS, the unexpected curtailment of Federal funds in fiscal year 1959 has had further discouragement added by another curtailment of up to 25% for fiscal year 1960 although the urgency of the problem has not diminished; and

WHEREAS, the brucellosis eradication program planned for appreciable accomplishment during the current fiscal year must now be delayed in many counties in several states wherein the essential preliminary steps had been initiated; and

WHEREAS, the postponement of adequate federal participation will promote loss of confidence and increase maintenance costs over a longer period; and

WHEREAS, curtailment of the eradication program will create a hardship on owners of cattle who move their animals interstate;

Now, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that the second session of the 86th Congress, convening in January 1960, be requested to take immediate and active steps to perfect the passage of a supplemental urgency appropriation of approximately $3 1/2 million dollars for continuation of the brucellosis eradication program on an active and progressive basis and in keeping with the assurances of federal cooperation when the program was offered to the states; and

BE IT FURTHER RESOLVED that copies of this recommendation be forwarded by each Commissioner, Secretary, or Director of Agriculture to his Senators and Congressmen with a letter explaining the effects of the reduction of current appropriation on the program in his state; and the Secretary of this Association forward a copy of this resolution to the Secretary of Agriculture and to the Bureau of the Budget.

RESOLUTION NO. XXXIII—VESICULAR EXANTHEMA

WHEREAS, vesicular exanthema, which has cost the swine industry of this country several million dollars, appears to have been eradicated; and
WHEREAS, it is generally agreed that cooking of garbage has been responsible for the eradication of this disease; and
WHEREAS, with the announcement of the eradication of vesicular exanthema there may be a tendency to relax the enforcement of the garbage cooking laws and regulations in the various states;
Now, THEREFORE, BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that it is extremely important that state and federal agencies continue to inspect garbage feeding premises and not abandon the enforcement of garbage cooking laws and regulations so as to prevent the spread of said disease.

RESOLUTION XXXIV—ANAPLASMOSIS

BE IT RESOLVED, by the National Association of State Departments of Agriculture assembled in convention at Nashville, Tennessee, October 19-23, 1959, that it request its Executive Committee to review with the Agricultural Research Service the problem of anaplasmosis; and
BE IT FURTHER RESOLVED, that the Executive Committee urge additional funds be made available to carefully carry out further research work and field trials for the control of anaplasmosis.

The National Association of State Departments of Agriculture discussed at its meetings and through its standing committees all of the phases of agricultural production and marketing. On the final day, the Association passed forty resolutions. The importance of animal diseases and their control in the minds of the commissioners was evidenced by the fact that, of the forty resolutions passed, fourteen dealt with this subject.
REPORT OF COMMITTEE ON BIOLOGICS AND PHARMACEUTICALS


The introduction of the 1958 report stated, "Historically the Committee on Biologicals and Pharmaceuticals has presented a report covering new or significant agents which have become available as implements in the hands of those individuals or groups concerned with the health and production of the livestock of our nation." Basically then, this has represented a progress report on categorized pharmaceuticals or biologics made available during the past year.

It is the opinion of your Committee that the mere reporting of such products does not constitute a progressive or constructive report, since information relating to such products—either biological or pharmaceutical is readily made available to the public at time of release.

It is the opinion of your Committee that we should be more concerned with the standards by which biologicals and pharmaceuticals are produced as well as their efficacy when used in accordance with manufacturer's instructions.

BIOLOGICALS

Your Committee believes that a standard order of procedure should be established by the Agricultural Research Service to evaluate all biologics prior to their release for use. Such a practice will assure greater protection to the welfare of the livestock industry.

In the field of infectious, transmissible diseases, vaccines have played a vital role. The philosophy for the future utilization of such products will continue at an increasing tempo. However, the justification for their utilization must be predicated on their efficacy, safety and promotion of a more economical livestock production.

To meet these standards your Committee recommends the following steps as a standard procedure in the development of commercial vaccines: The viral agent must produce illness and productivity loss in the natural host. By serial passage the virus is attenuated or modified to the degree that it will not produce signs of illness. There shall be preliminary safety tests in the natural host for signs of illness, transmissibility and reversion to virulence. There should be continued passage through the alien host to the highest level, together with determination of minimal immunizing dose. There should be laboratory standardization of the vaccine against specific measured infective doses of the agent to eliminate batch differences in the commercial dose. It should then be subjected to efficacy and safety tests in the field.
Your Committee believes that in the field of pharmaceutical production a standard order of procedure should be established by the appropriate federal agency. Such standard could incorporate philosophies to insure product efficiency as well as safety. Safety evaluations must be beyond mere toxicity to the patient or the animal, but in light of current events, should be evaluated for residual implications to the public health and welfare.

The relationship of chemical compounds or combinations of compounds to livestock health and production, should not be confined to the therapeutic use of such compounds to treat the single animal, but should be broadened to include herd or flock medication for the prevention of diseases. Beyond this, consideration must be given to the accidental contact by ingestion or external herbicides, insecticides, fertilizers and preservatives.

It is apparent that the fields of pharmaceutical and biologics have broader implications than formerly considered. Therefore your Committee recommends consideration be given to broadening the membership of this committee to include members of the pharmaceutical, biological and related disciplines such as chemistry, toxicology, entomology, nutrition, veterinary medicine and regulatory drug personnel, with continuing membership that rotates over a period of time, greater than one year, so that the continuity of effort may be maintained.

Because of the rapid changes in our present methods of livestock production and the challenge to control or eradicate diseases through the utilization of biologics, your Committee offers the following resolutions:

WHEREAS, the science of veterinary medicine has developed and is continuously developing new and revolutionary vaccines and other immunizing agents for the prevention and treatment of animal diseases, and

WHEREAS, new biological products resulting from such research are coming on the market for the protection and improvement of livestock and other domestic animals, including dogs and household pets, and

WHEREAS, the national interest demands that suitable standards be established and enforced to regulate the commercial production, sale and use of such products to assure livestock and animal owners, the dairy and meat industries, and other owners of domestic animals, including dogs and household pets, safe and potent products for the prevention and treatment of animal diseases.

Now, Therefore, Be It Resolved:

1. That the Congress be petitioned to authorize and finance an accelerated and adequate program of licensing and inspection to include the development, investigation, adoption and enforcement of standard procedures for evaluating the safety and potency of animal biological products, as they are developed and placed on the market.

2. That such program of licensing etc., be authorized through the existing units already designated for this purpose within Agricultural Research Service, United States Department of Agriculture.
In view of the ever increasing number of new viral agents which have been and continue to be isolated from various species of domestic animals, the Committee feels that there is a pressing need for a mechanism whereby these agents may be grouped and subsequently classified. It is recommended therefore that a Viral Research Committee be established as an aid to the individual investigator so that new agents may be checked as follows:

1. The antigenic analysis to be made on the basis of serum neutralization, the procedures for which should be standardized for all cooperating laboratories.

2. That prior to the reporting of a new viral agent the following broad criteria be thoroughly evaluated as regards the new agent:
   
   I  Antigenic Analysis  
   II  Biological characteristics  
   III  Pathogenesis and tissue tropism  
   IV  Physical characteristics  
   V  Chemical characteristics  

3. That the investigators be encouraged to exchange viral agents and appropriate antisera with as many cooperating laboratories as the Committee may interest in the program.

4. That the Committee be authorized to suggest cooperating laboratories and to propose the establishment of a suitable central repository for viral agents similar to that operated by the American Type Culture Collection.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION
COMMITTEE ON LEGISLATION

W. L. BENDIX, Chairman, Richmond, Virginia; T. C. GREEN, Charleston, West Virginia; T. J. GRENAN, Providence, Rhode Island; R. A. HENDERSHOTT, Trenton, New Jersey; J. A. McCALLAM, Washington, D. C.

ANNUAL REPORT 1959

The Committee on Legislation of the United States Livestock Sanitary Association, through its chairman, was actively engaged in behalf of the Association during the term of the First Session of the Eighty-Sixth Congress. The Committee wishes at this time to acknowledge the fine cooperation it received from the Washington representative of the American Veterinary Medical Association and from the Executive Committee of the National Association of State Departments of Agriculture. The Committee on Legislation, also through its chairman, worked very closely through the year with the Advisory Committee to the Agricultural Research Service. Experience has indicated that close liaison between these two committees of this Association, namely the Advisory Committee and the Committee on Legislation, makes for greater effectiveness of the work of both committees and has proven a most desirable arrangement.

The federal appropriation for continuation of the accelerated brucellosis program for the fiscal year 1960 required more concentrated effort this year than in any year since its inauguration. Factual data submitted to the Advisory Committee to the Agricultural Research Service indicated that the $20,000,000.00 per annum figure was just about the minimum necessary to carry this program to completion. The Animal Disease Eradication Division prepared a chart showing the costs of new county certification work at the rate the program has been operating, plus the added cost of recertification of those counties already completed. This graph showed clearly that as more and more counties were added to the certified list and came up for recertification, the annual cost gradually rose to a point estimated at close to $15,000,000.00 a year to maintain all the counties in the country after certification is completed. To reduce the funds available materially before all counties are certified simply means that as more and more counties come up for recertification, a slow-down of the work in progress in counties not yet certified is inevitable; and when funds are curtailed close to $15,000,000.00 per year, the whole program of new work slowly will grind to a halt as the fixed charges catch up with the available funds.

You are all aware that we were unsuccessful in convincing the Congress of the importance of this fact. The House of Representatives passed a $15,000,000.00 appropriation. This Association in conjunction with the American Veterinary Medical Association and the Executive Committee of the National Association of State Departments of Agriculture persuaded the
Senate to raise this to $17,500,000.00. In the Senate-House conference, a compromise was reached at $16,250,000.00, which was passed by both houses and signed by the President on July 8, 1959. The chairman of the Legislative Committee notified all chief livestock sanitary officials of the facts when this item went to the conference committee and urged them to do what they could do to persuade the conferees to adopt the Senate figure. The Legislative Committee wishes to thank all of the chief livestock sanitary officials and others who responded so promptly, even though we did not achieve our aim.

The National Association of State Departments of Agriculture, at its annual meeting in Nashville, Tennessee, October 19-23, 1959, adopted a resolution instructing its Executive Committee to proceed immediately upon the convening of the Second Session of the Eighty-Sixth Congress in January to seek a $3,500,000.00 emergency appropriation for the balance of the fiscal year 1960 for this work.

 Probably the most controversial piece of legislation directly affecting this Association introduced into the Congress this year was Senate Bill 864, introduced by Mr. Humphrey, and House Bill 7317, introduced by Mr. Fisher as a companion measure. This legislation was entitled "A Bill to Provide Greater Protection Against the Introduction and Dissemination of Diseases of Livestock and Poultry, and for Other Purposes." A good deal of this bill is an effort to clarify the language of authority already in the hands of the Secretary of Agriculture to control communicable diseases of livestock and poultry. New features of the bill would grant the Secretary authority to seize and dispose of diseased or exposed animals in interstate commerce, or such animals that had been moved in interstate commerce. There was little controversy regarding this provision, although the chairman of the Legislative Committee was successful in introducing an amendment to this section which served only to clarify the intent and in no way changed the provision. Currently the Secretary's authority is limited to actual proceedings against those persons who violate the federal interstate or import regulations, with no authority to seize and dispose of the animals involved. Certainly there can be little argument opposed to this added authority. In actual fact, the seizure and proper disposal of the animals in question is more important than any fine imposed against persons transporting such animals.

The section of the bill that aroused the principal controversy was the one dealing with extending the Secretary's authority to seize and dispose of animals, the carcasses of such animals, and any products and articles that were related to such animals on any premise in the United States, whether or not interstate or foreign commerce was or had been involved. The bill proposed to limit the Secretary's authority to do this to the existence of an extremely dangerous disease and would require the declaration of an extraordinary emergency. As originally drawn, the bill provided that the Secretary must notify the state in which he intended to take such action, but would not be bound by consent or approval of any such state. Here again, the chairman of the Legislative Committee succeeded in introducing an amend-
ment to this section which prohibited the Secretary from taking this action unless it was determined that the state or states involved were not taking adequate measures under the police powers inherent within them. Here against, the amendment was not intended to weaken the section, but merely to clarify the Congress’s intent and to afford the states an opportunity to act without interference from the federal government should they so desire. The bill was passed by the Senate with amendments, but was tabled in the House subcommittee as a result of rather strenuous opposition by certain industry groups. The United States Livestock Sanitary Association was not in a position to take any official action, although in the committees of both the House and the Senate the question was asked as to whether or not the Association had anything to say. When the chairman of the Legislative Committee sent to each livestock official in the nation a copy of this bill, along with a detailed analysis of its provisions prepared by the Department of Agriculture, and asked for guidance, the replies received were so divided that no official statement by the United States Livestock Sanitary Association was possible.

Numerous other bills were introduced into the Congress, most of which failed of passage, dealing with the establishment of a national wild life disease laboratory, the erection of a land boundary fence and its maintenance between the United States and Mexico, and to permit the Department of Agriculture to cooperate with the meat-inspection services of the various states, etc. The latter bills (H. R. 8951, H. R. 8954, and H. R. 9187) authorize the Secretary to recognize the meat-inspection services of the various states when and if they are doing a job comparable to the Federal Meat Inspection Service, may be worthy of some mention here. The greatly expanding demand for meat-inspection services has for some years kept the Federal Meat Inspection Service operating on emergency appropriations and never quite being able to staff and service all the plants qualified and applying. It is becoming year by year increasingly more difficult to get adequate appropriations in the annual appropriation bill for this service. The same groups year after year go to the Congress with the same story and achieve about the same result, which is never quite enough. It would seem that the bill which would authorize the Secretary to make use of existing state meat-inspection services to supply this demand at no additional cost to the government would be desirable and would provide for considerable extension of actual meat inspection in the public interest. It would also seem that were such recognition authorized, it would tend to greatly improve the quality of state meat-inspection operations and to bring them into a comparable position with the federal service. Strangely enough, the general opinion in the Department of Agriculture seems to be in opposition to these bills or any similar legislation.

RECOMMENDATIONS

This Association will recall that the report of the Committee on Legislation of 1958 dealt with the difficulty encountered at times when legislation was introduced into the Congress and the Committee on Legislation through its
chairman had to take a position in the absence of any definite prior action by this Association. The Executive Committee of this Association recognized this difficulty, but declined to accept the 1958 recommendation of the Legislative Committee for a solution. The Legislative Committee was directed to give further thought to this and to make a recommendation again this year. Most of the national organizations with problems of this kind leave the decisions to their executive committees when the associations are not in session, and grant the executive committee the authority to act for them. Most executive committees, however, are relatively small bodies, whereas the Executive Committee of this Association has representation from all 50 states, and now has under consideration a further enlargement. This would not be a practical or a workable solution then in this case. At the beginning of this report, mention was made of the close liaison between the chairman of the Legislative Committee and the work of the Advisory Committee for the Agricultural Research Service. The work of these committees is frequently parallel and closely intermingled. The Advisory Committee from time to time calls on the Legislative Committee to carry its recommendations to the Congress. The Agricultural Research Service from time to time takes up its needs, both financial and legislative, with both committees. This, then, prompts the following recommendations:

1. It is recommended that this Association combine the Committee on Legislation and the Advisory Committee to the Agricultural Research Service into a single committee, to be known as “Committee on Federal Programs and Policy.” It is further recommended that this Committee be composed of the officials of the Association and that the First Vice-President serve as its chairman. In addition, it is recommended that the Committee include representation elected from each of the four regional associations, made up of the livestock sanitary officials and such additional membership (not to exceed four) as the President may from time to time appoint. It is further recommended that this Committee elect one of its members to serve as legislative representative, and that such legislative representative shall take only such action before the Congress as has three-fourths approval of the Committee. It is recommended that the Secretary-Treasurer of this Association act as secretary of this Committee and that he be instructed to keep complete minutes of all meetings and actions, which shall become a part of the proceedings of this Association and be published annually.

2. It is recommended that this Association instruct the “Committee on Federal Programs and Policy” to make every effort to have the current appropriation for brucellosis eradication increased by the sum of $3,500,000.00 by the Second Session of the Eighty-Sixth Congress, for use in the second half of fiscal 1960.

3. The Committee recommends that this Association approve and support legislation authorizing the Secretary of Agriculture in his discretion to cooperate with the meat-inspection services of the several states, using these services as a means of broadening meat-inspection services of the several states to supply approved meat and meat-food products in interstate commerce.
4. As previously stated, the basic reason for the wide difference of opinion regarding Senate Bill 864 is centered around the authority sought for the Secretary of Agriculture in case of an extraordinary emergency to seize and dispose of animals and products on any premise in the United States. Without this provision, the rest of the bill, including authority for the Secretary to seize and dispose of animals that are or had been in interstate or foreign commerce, would probably receive widespread support and pass without opposition. The Committee recommends that this Association go on record urging the Department of Agriculture to prepare and execute with each of the several states memoranda of understanding and agreement dealing with the handling and eradication of disease conditions for which an extraordinary emergency has been declared or would be justified. These agreements should parallel the existing agreements for the routine disease-control efforts jointly carried out by the federal government and the states, wherein the duties and responsibilities of each are clearly defined. This would allow each state to clearly understand what its responsibilities would be under such conditions and to arrange its forces and its authority so as to be able to fully meet them. If this were done, it is felt that legislation could then be sought and supported both by the states and by industry that would give the nation adequate protection under conditions of extraordinary emergency should any state or states violate or fail to fulfil the provisions of their agreements.
REPORT OF COMMITTEE ON PUBLIC RELATIONS

J. E. STUART, Chairman, Sacramento, California; J. D. DeMATTEI, San Francisco, California; T. C. GREEN, Charleston, West Virginia; R. L. KNUDSEN, Washington, D. C.; A. K. KUTTNER, Salt Lake City, Utah; D. L. LICHTY, West Palm Beach, Florida; R. J. SCHROEDER, Los Angeles, California.

Mr. President, Members of the Association, and Invited Guests: Your Committee registers the same complaint that previous committees have made—that papers are not submitted in time to enable the Committees to adequately release publicity therefrom. Perhaps we should take a different approach to this problem. It seems doubtful whether the present system will ever prove satisfactory; therefore, your Committee recommends that publicity should be a primary responsibility of the Secretary of the Association and that the publicity released should be on a year round and continuous basis. By doing this we could save at least one time-consuming step of handling the material, and thereby expedite the distribution of informative material pertaining to meetings, papers and the like to the press. We believe that a continuous program should be instituted of releasing regularly (perhaps monthly) a news release to the agricultural press, trade journals and veterinary journals.

We repeat the proposal of the 1956 Committee that consideration be given to placing a reference copy of each year’s proceedings in the hands of the principal farm and livestock publications and radio and television farm directors.

In general, the livestock industry, animal husbandry and veterinary students, and even the veterinary profession, are not informed regarding the functions of the Association.

In addition to a year round publicity program, the Committee recommends that an informative brochure be prepared, explaining what the Association is and what it does. Adequate distribution of this should be made, especially to students and the agricultural press, and copies for distribution should be furnished to all members of the Association for their convenience in informing others about the Association.
REPORT OF THE COMMITTEE ON REGULATORY EDUCATION


Progressively more emphasis is being placed on regulatory veterinary medicine by our professional schools and colleges. Those concerned hope that further emphasis on regulatory control of animal diseases will strengthen and broaden the veterinarian's responsibility and effectiveness in this phase of veterinary medicine. The curricula of high schools and colleges of veterinary medicine are continually being changed, closer relationships being developed in all areas, and especially among those directly related to regulatory work. Modern-day veterinary medical graduates are competent to carry out satisfactorily not only the various applied aspects of disease control and meat hygiene, but the entire area of food products of animal origin. If the veterinary profession is to assume and strengthen its proper position in the "household" of medical sciences, all areas should be broadened. Such qualification is particularly important in animal disease regulatory work, because, in many instances, the veterinarian so engaged contacts and deals with those in other health professions. Regulatory veterinary medicine can better accomplish these desired results if the individuals, working or planning to work in this field, obtain some formal advanced training beyond the doctor of veterinary medical degree.

When circumstances permit, the Department of Agriculture allows some of its veterinarians to do graduate work. This is possible while we are carrying out certain duties in cooperative programs at the schools and colleges of veterinary medicine. Continued development of the program will do much to strengthen regulatory veterinary medicine, both generally and specifically.

The Committee does not recommend any specific changes in the professional curriculum. However, it urges further encouragement to veterinarians who avail themselves of opportunities for specialized advance work in order that their proficiency may be increased. It would seem appropriate for this Committee to encourage the development of more extensive training in regulatory veterinary medicine, and to report on progress at intervals of two or three years, rather than annually.
REPORT OF THE COMMITTEE ON RESOLUTIONS


The following resolutions are presented for your action:

RESOLUTION NO. I—EMERGENCY FUNDS FOR FEDERAL-STATE BRUCELLOSIS ERADICATION PROGRAM

WHEREAS, an accelerated brucellosis eradication program was encouraged by federal authorities and by Congressional action through allocation of $24,000,000 for such purpose in 1954, followed by comparable allocation in 1960; and

WHEREAS, many states in reliance thereon stepped up their programs commensurately, resulting in primary certification in 21 such states, although some states, through no fault of their own, could only adopt the program in slower stride; and

WHEREAS, the unexpected curtailment of federal funds in fiscal year 1959 has had further discouragement added by another curtailment of up to 25 percent for fiscal year 1960, although the urgency of the problem has not diminished; and

WHEREAS, the brucellosis eradication program planned for appreciable accomplishment during the current fiscal year must now be delayed in many counties in several states wherein the essential preliminary steps had been initiated; and

WHEREAS, the postponement of adequate federal participation will promote loss of confidence and increase maintenance costs over a longer period; and

WHEREAS, curtailment of the eradication program will create a hardship on owners of cattle who move their animals interstate;

NOW, THEREFORE, BE IT RESOLVED, that the Executive Committee of the United States Livestock Sanitary Association assembled in Convention in San Francisco, California, December 16-18, 1959, urgently request the second session of the 86th Congress appropriate approximately $3,500,000 or such sums as are necessary to provide for a continuation of the Brucellosis Eradication Program on an active and progressive basis in keeping with the assurances of federal cooperation when the program was offered to the states; and

BE IT FURTHER RESOLVED, that copies of this recommendation be forwarded by each State Regulatory Official to his Senator and Congressman with a letter explaining the effects of the reduction of current appropriation on the Brucellosis Program in his state; and the Secretary of Agriculture, and to the Bureau of the Budget.
RESOLUTIONS

RESOLUTION NO. II—SHEEP SCABIES

WHEREAS, the number of flocks of sheep reported as affected with psoroptic scabies continues to increase; and

WHEREAS, the continued existence of scabies in the affected states constitutes a hazard for large areas in which scabies has been eradicated;

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Association request the Secretary of the United States Department of Agriculture to develop a national federal-state cooperative project for the eradication of sheep scabies; and

BE IT FURTHER RESOLVED, that this Association urge all state livestock sanitary officials to cooperate in such a project.

RESOLUTION NO. III—SCRAPIE RESEARCH

WHEREAS, the satisfactory control of scrapie depends upon the results of further research; and

WHEREAS, the sheep industry has requested that the United States Department of Agriculture undertake research on scrapie; and

WHEREAS, the Agricultural Research Service does not have immediately available adequate and safe facilities for such research in the United States, and

WHEREAS, very extensive research on scrapie is in progress in Britain, to which the United States is now making a small contribution;

BE IT RESOLVED, that the United States Livestock Sanitary Association request the Secretary of the Department of Agriculture to increase the contribution of personnel and funds to the research projects of the British research institutions, as the most economical and effective way to accelerate the production of results from scrapie research.

RESOLUTION NO. IV—FOOT-ROT

WHEREAS, Foot-Rot of sheep is more or less prevalent in the sheep producing area of the United States, particularly in farm flocks, but to some extent in range sheep; and

WHEREAS, this disease has become a serious handicap to farm flock producers in some areas; and

WHEREAS, there are practical procedures by the use of which any flock of sheep can be freed from foot-rot and kept free; and

WHEREAS, the only source of the infection is an infected sheep;

BE IT RESOLVED, that the United States Livestock Sanitary Association go on record as recommending to all state and federal regulatory officers (1) That foot-rot of sheep be recognized as a reportable disease; (2) That infected flocks be quarantined; and (3) That the sheep be treated under supervision and held in quarantine until the Livestock Sanitary Authority determines that the disease has been eradicated in the flocks so treated.
RESOLUTION NO. V—DEALING WITH THE ST. LAWRENCE SEAWAY

WHEREAS, the opening of the St. Lawrence Seaway has created problems in the field of port inspection; and

WHEREAS, there is a critical shortage of funds and personnel at the federal level to furnish sufficient inspection at ports along this seaway; and

WHEREAS, the health of the livestock is endangered by these conditions;

NOW, THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Association, through its legislative and advisory committees, use all means at its disposal to see that sufficient federal funds are made available at these ports to protect the livestock industry of this country.

RESOLUTION NO. VI—REFERRING TO MEANS OF EXPANDING RESEARCH IN ANIMAL DISEASES

WHEREAS, the estimated annual toll among farm animals due to diseases and parasites in 2,700,000,000 dollars; and

WHEREAS, 54 percent of total farm income is derived from livestock and poultry; and

WHEREAS, only 5 percent of state and federal agricultural research operating funds are devoted to animal disease and parasite problems; and

WHEREAS, laboratory facilities requiring adequate isolation provisions for both the laboratory and farm animals are among the most expensive facilities used for research and regulatory programs relating to agriculture; and

WHEREAS, many states maintain such laboratories, staffed with talented scientists, in connection with their livestock and poultry disease programs; and

WHEREAS, the research activities of these State Veterinary Laboratories could be greatly expanded with relatively little additional cost; and

WHEREAS, cooperation is in the use of expensive facilities and the services of talented scientists promotes economy and efficiency;

NOW, THEREFORE, BE IT RESOLVED, by the United States Livestock Sanitary Association, meeting in San Francisco, California, December 15 to 18, 1959, that this Association work with the Agricultural Research Service to expand research in animal diseases in State Veterinary Laboratories in a cooperative manner similar to that now in effect between the Agricultural Research Service and the various State Agricultural Experiment Stations; and

BE IT THEREFORE RESOLVED, that the Committee on Program and Policy of this Association be and is hereby directed to consider this matter and work out effective means for the implementation of this Resolution.
REPORT ON THE PROPOSED REVISION OF THE
CONSTITUTION AND BY-LAWS

Dr. A. K. Kuttler

Salt Lake City, Utah

Mr. Chairman, Members and Guests of the United States Livestock Sanitary Association. I had not anticipated being called upon to give this report. However, I can assure you I would have been deprived of a great deal of gratification if I had not been afforded this opportunity. Under the Constitution it is necessary to publish the Proposal, a year before it is ratified. The change deals with the livestock industry representation on the Executive Committee of this Association, and simply provides that there will be two industry representatives from each of four extension service districts. It was thought well to divide it on this basis. It provides for the nomination of two farm representatives from each of the four agricultural extension service sections of the United States, namely the Northeast, the South, the North Central, and the West. This proposed Amendment was printed in the Sixty-Second Annual Proceedings of this Association and has at this meeting been approved by the Executive Committee unanimously. I should like to entertain a motion to adopt this change in the Constitution and By-Laws of the United States Livestock Sanitary Association by members assembled at this convention.

Dr. L. R. Noyes, of Texas: I move that the Constitution and By-Laws of this Association be amended as the Executive Committee approved last evening.

Dr. W. F. Fisher, of Nevada: I wish to second the motion.

President Francis D. Buzzell: Are there any questions? Are you ready for the question? Those in favor of the amendment make it manifest by saying “Aye.” Opposed? The “Ayes” have it; so ordered.
In the late summer it was decided that Animal Disease Eradication Division would publish the successor to Circular 1, and I was given the task of compiling it for publication.

The recommendations of the United States Livestock Sanitary Association Laws and Regulations Committee were studied, and during the meeting of the American Veterinary Medical Association in Kansas City I met with members of this Committee to discuss publication of the book.

It was decided to make this a loose leaf book, divided into various sections. The first section is to be the requirements of the individual states for entry of livestock and poultry into their respective states, these laws and regulations to be compiled, approved and certified by the chief livestock sanitary official of each state. The first section of our book is printed on blue paper for easy reference.

The second section is our federal regulations governing the interstate movement of livestock and is a reprint of Title 9, sub-chapters B & C of Chapter 1, Code of Federal Regulations. This section is printed on white paper for quick reference.

The third section is composed of regulations of the Animal Inspection and Quarantine Division and has to do with import and export of animals into and from the United States. This section is printed on salmon-colored paper for quick reference.

The last section contains the Canadian import regulations for entry of livestock into Canada. This section is printed on green paper for easy reference.

It is planned to print sufficient copies that all interested persons might have a copy. In order for it to be an up-to-date, effective, complete book of Regulations and Laws, it will be necessary to keep it up-to-date with loose leaf changes and revisions. This is planned in the following manner:

The supply for each state will be maintained in the office of our veterinarian in charge in each state, who will maintain a complete list of every person receiving a copy.

It is planned to review, revise and correct the book at least every three months. The corrections will be printed on the same colored paper as the original and numbered to replace sections to be corrected. These will be mailed to each state to supply the master list in each state. They will be sent out from our veterinarian in charge's office to the holder of each book. It is hoped in this way to keep the book up-to-date and not becoming obsolete.

I am sure you will find it very handy for reference purposes and see that it fills a very great need.
REPORT OF COMMITTEES ON LAWS AND REGULATIONS

A. P. SCHNEIDER, Chairman, Boise, Idaho; J. W. GREEN, Indianapolis, Indiana; T. C. GREEN, South Charleston, West Virginia; F. X. HONSINGER, Juneau, Alaska; DAVID IBSEN, Little Rock, Arkansas; M. D. MITCHELL, Pierre, South Dakota; H. J. ROLLINS, Raleigh, North Carolina; A. L. SUNDBERG, Des Moines, Iowa; F. E. ZIEGENBEIN, Lincoln, Nebraska.

As you know this Committee has been keenly concerned as to the possibility of unification of entrance requirements for various classes of livestock, and wishes to reiterate that each livestock regulatory official give the utmost attention to the possibility of uniform requirements for the interstate movement of livestock of the various classes.

It appears that the first step in this direction might be uniformity for various blocks or groups of states that have similar problems. These groups might consist of states that deal primarily with imports or exports of livestock, secondly, it might be groups on the basis of being predominantly dairy or beef. However, to start, it appears that similar geographical areas of the United States might be the first step in which to start.

Your Committee continues to urge the adoption of the following regulation within each state covering the admittance of sheep and goats, as it appears we are making good progress in the control and eradication of this disease.

The regulation as approved last year reads as follows: "Sheep originating in a state in which scrapie is known to exist or has existed within 42 months prior to shipment, in addition to being accompanied by usual health certificate, must bear special notation thereon, or, a separate statement properly attested or certified to by the livestock sanitary authorities of the state of origin, to the effect that such sheep are not the progeny of scrapie exposed or scrapie infected sheep, or have been exposed to scrapie exposed or infected sheep."

Your Committee is pleased to report that a meeting was held during the year at the American Veterinary Medical Association at Kansas City, on August 25, 1959. This meeting was attended by Dr. F. L. Herchenroeder, of the United States Department of Agriculture, Agricultural Research Service, with regards to publication of a new issue of the United States Livestock Sanitary Association Circular No. 1, entitled "Health Requirements Governing Admission of Livestock." The purpose of this meeting was to hear the facts concerning the reissuing of this circular in a loose leaf form, and in bringing the various state requirements up-to-date.

The latest report from the United States Department of Agriculture, is that all the states have furnished their regulations, and that loose leaf circulars will be available for distribution shortly after January 1, 1960.
REPORT OF COMMITTEE ON PARASITIC DISEASES, UNITED STATES LIVESTOCK SANITARY ASSOCIATION

J. L. Hourigan, Chairman, Washington, D. C.; R. L. Cuff, Kansas City, Missouri; Dr. V. D. Chadwick, Jackson, Mississippi; Dr. W. M. Thompson, Phoenix, Arizona; Dr. C. E. Kord, Nashville, Tennessee; Dr. F. R. Kutz, Columbus, Ohio.

STATUS OF SCREWWORM ERADICATION PROGRAM

The basic concept leading to the screwworm eradication program in the Southeastern States along with details involving basic research on the habits and development of rearing techniques of the screwworm fly Callitroga hominivorax (Cqrl) were discussed in the report submitted to your Committee in 1958. Preliminary field tests to improve techniques and successful launching of an all-out eradication program in the southeast in mid 1958 were also discussed in that report.

Fly production operations were begun on schedule in July 1958 at the screwworm rearing facility at Sebring, Florida. The production goal of 50 million sterile flies per week was reached within a relatively short period of time providing an ample number of flies to release from small airplanes over a maximum area of 85,000 square miles in Florida, Georgia, and Alabama.

The overwhelming number of released flies eventually reduced the native population in the eradication area to a very low level with the exception of a relatively small three-county area near the southern tip of Florida. Here, in November, and December, 1958, and in January and February, 1959, an unusual situation was encountered when attempts to eradicate this last stronghold of native flies seemed for a time ineffective. In Broward county it was necessary to release as many as 9,000 sterile males per square mile per week before achieving desired results. In addition to those fly releases, range riders were employed to constantly inspect and treat wounds and the navels of new-born animals to further reduce the native fly population. Fly traps were placed at strategic points within the "hot spot" area to accomplish a further reduction of native flies. Sterile male releases were also made on the ground to build up the sterile male population in likely screwworm hideouts.

Through the use of these concentrated eradication measures the last known screwworm larvae in Broward county were found and destroyed on February 19, 1959.

The production and release of approximately 50 million sterile flies per week continued throughout the remainder of the fiscal year with dissemination rates of 100 to 600 sterile males per square mile throughout Florida and parts of Alabama and Georgia. In spite of the intensive efforts of field personnel, additional cases of screwworms were not found within the fly
release area until June 17, 1959, when larvae were found in a dehorning wound in a heifer in Highlands county near the center of Florida. Fly releases and inspection efforts were immediately stepped up in this vicinity.

Continued negative findings in the field thereafter prompted program officials to test the effectiveness of the eradication effort. In late July the release of sterile flies was discontinued in that part of Florida which lies south of Lake Okeechobee, a total area of approximately 10,000 square miles and one in which the last concentrated native fly population had been found.

The force of livestock inspectors in the area was doubled and livestock owners were urged to cooperate even more closely in reporting any suspicious findings.

The test indicated at its conclusion that the native fly population in that part of Florida, had been destroyed. It was then decided to cease the production and release of flies throughout the eradication area, but to continue full-scale detection operations in the field.

The fly production establishment at Sebring was closed and placed on a stand-by basis in November. To accomplish this it was necessary to destroy the fly colony maintained for egg production purposes, sterilize all remaining screwworms as they reached the proper pupal stage, and finally to spray the interior with synergized pyrethrum, then fumigate with TEPP and DDVP, organic phosphate pesticides with short residual toxicity.

Approximately 3 billion sterile flies were produced and released during the 17 months of operations. In this process, 6 1/4 million pounds of meat were utilized.

A fly colony for reactivating the rearing operation, should the need for such action arise, is being maintained at a United States Department of Agriculture laboratory at Kerrville, Texas.

Thirteen inspection stations located along the eastern boundaries of Arkansas and Louisiana are open on a 24-hour, seven-day-week basis to provide for inspection and treatment of animals entering the eradication area. Since the operation began in 1958, more than 300 thousand animals have been checked at these stations for evidence of screwworm infestation and of this total, 14 animals were found infested and treated to rid them of screwworms before continuing to destinations in Georgia, Florida, Alabama, South Carolina, and Mississippi.

During the period August-November 1959, approximately 175 cases of screwworms were found in eight counties located along the Mississippi River in Mississippi at the same time screwworms were being found on the west side of the river in Louisiana and Arkansas. Almost 50 thousand animals in the vicinity were treated with a pesticide to prevent spread due to population buildups. Establishment of an intensive animal inspection program in the area facilitated control of the outbreak and enlisted the aid of livestock owners in preventing dissemination. By early November cold weather had reduced the incidence of infestation to only an occasional case. During the fall of 1958 a similar, but less severe outbreak, occurred in the same vicinity.

Your Committee recognizes the importance of maintaining adequate procedures to prevent reintroduction of screwworms into the Southeastern area
and urges that all practical means be taken to prevent reintroduction of screwworms into the area where the eradication program has shown excellent promise eliminating this pest.

A screwworm survey was made in the Southwestern United States and northeastern Mexico during 1959 to determine incidence, relative abundance, and overwintering areas, and to obtain other pertinent information relative to the screwworm problem.

The survey revealed that screwworms remain active throughout the winter months in parts of Texas, Arizona, and California, and in all of northeastern Mexico. The total area where screwworms overwinter in the Southwestern United States approximates 150,000 square miles. The overwintering area in adjacent Mexico totals several hundred thousand square miles.

Losses due to screwworms in these areas are estimated to be somewhat less per unit of area than they were in the Southeastern states.

No natural barrier was found in Mexico that would prevent the migration of flies and subsequent reinfestation of uninfested areas.

Information obtained from the survey indicates that in the absence of a natural barrier, the success of any eradication program using the sterile male technique would be dependent upon developing means to establish and maintain an artificial buffer zone of sufficient magnitude to prevent reinfestation of any area that might be freed of screwworms.

An eradication program, therefore, would not be feasible without a continuing program to prevent reinfestation.

The estimated cost of an eradication program is $100.00 per square mile per year, plus the initial cost of buildings, equipment, and other facilities which will approximate $2.1 million per 80,000 square mile unit.

RESISTANCE OF INSECT PESTS TO PESTICIDES

The resistance of insect pests to pesticides was first noted about 40 years ago. During the past 12 years, i.e. with the advent of DDT and other chlorinated hydrocarbon pesticides, insect resistance to pesticides has become a serious problem throughout the world. This problem is not as acute in the United States as in some other countries, but it is increasing and must be viewed with real concern.

Most of the publicity on insect resistance to pesticides has been from the standpoint of public health where no less than 50 arthropod species have developed resistance to chlorinated hydrocarbon or organophosphorus pesticides. Much less publicity has been given to pests of livestock and poultry, but it is important to remember that some species of insects of public health importance are also vectors of animal diseases. House flies, numerous species of mosquitoes, brown dog ticks, cat fleas, and goat lice have been found to be resistant to one or more classes of pesticides. Many of the resistant species can be controlled with the organic phosphorus insecticides, but several species of mosquitoes and the house fly have already become resistant to these pesticides.
The substitution of more powerful pesticides from year to year is not a satisfactory answer to the insect resistance problem. More attention should be given to developing new types of chemicals and methods of application with a minimum of hazard to man, animals, wildlife and beneficial insects. More intensive study of biological and cultural control methods may offer a solution to the control of some pesticide resistant insects.

**SYSTEMIC PESTICIDES**

A systemic pesticide for livestock may be defined as a chemical which, when administered to an animal as a spray, dip, injection, bolus, drench, or feed additive, is absorbed into the body tissues, either in its original form or as a metabolite, and is toxic to susceptible parasites feeding on such tissues.

Most of the experimental work with systemics during the past year concerned the control of cattle grubs; however, numerous observations were made on the control of lice, horn flies, screwworms, fleas, ticks, fleeceworms, and horse bots. The cost of treatment ranged from fifty cents to $1.50 per animal depending on the size of the animal and the insecticide used. Systemics showing the most promise are Co-Ral, ronnel, Ruelene and dime-thoate. The results of various tests have been inconsistent. The control of cattle grubs ranged from 0 to 100%. Some of the failures were presumably due to (1) improper dosage, (2) improper application, (3) improper time of treatment, and (4) the physiological condition of the treated animal.

Systemic pesticides show promise of effectively controlling many ectoparasites, and some endoparasites. Tests are being continued in many states, mostly with ronnel, Co-Ral, and Ruelene.

**THE BITING LOUSE OF SHEEP**

The biting louse of sheep, *Bovicola ovis*, is quite common throughout the country and has recently become very troublesome in western range flocks.

Although the biting louse is the least harmful of the species attacking sheep, it does cause considerable irritation and attempts made by the infested animals to gain relief by rubbing, scratching, and biting result in the soiling, matting, and breaking of the fleece, thereby reducing its value and usefulness. These increasing losses to the wool producer warrant giving more attention to a determined effort toward louse control.

Effective insecticides are toxaphene, lindane, DDT, methoxychlor and chlordane.

**CORRELATION OF PESTICIDE RESEARCH WORK AND FIELD WORK**

During this past year the attention of your Committee has been focused on the problem of improved acaricides, particularly those used in scabies and tick eradication programs. The problem involves efficacy of acaricides, their toxicity to animals, tissue residues, and development of suitable vatside testing procedures. The urgent need for definitive information continues to plague both research and field workers.
Your Committee recognizes that an area exists between purely research interests and broad field application that requires carefully coordinated activities of research and field workers if safe and practical procedures are to be found.

Research in blazing the trail, must work its way through sometimes discouraging experiences, before formulating a recommendation. Often an insufficient number of animals is available to research workers and considerable additional work under actual field conditions is necessary prior to general use of a pesticide. Your Committee recommends that concerted efforts be made to more closely correlate research on pesticides with field control and eradication problems. Field workers can make a worthwhile additional contribution in arranging for the use of far greater numbers of test and control animals under varying conditions. The results obtained through field trials could substantially add to the practical evaluation of research findings and pave the way to safer and more rapid general use of acaricides.

PHENOTHIAZINE AND DICTYCID

Your Committee has received reports concerning the use of phenothiazine and dictyicide for the control of internal parasites in domestic animals. Some workers have questioned the effectiveness of these drugs while others believe them to be of sufficient value to continue their use. We feel that the Association should encourage additional research on these chemicals to determine their efficacy under field conditions, and to develop new chemicals so urgently needed for parasite control.

SHEEP AND CATTLE SCABIES

Your Committee wishes to make particular mention of sheep and cattle scabies. Last year psoroptic cattle scabies was reported in 27 herds in six states, the greatest number reported since 1954. We urge that every effort be made to locate and eliminate all foci of this disease. Psoroptic sheep scabies was reported in 736 flocks in 24 states and in 209 lots of sheep at 18 stockyards.

Your Committee urges that a Sheep Scabies Eradication Program be vigorously pursued. This should include prompt application of both state and federal regulations when needed to prevent additional spread of the disease, increased state and federal funds to carry out an accelerated eradication program and greatly intensified eradication efforts in areas where the disease is endemic.
REPORT OF COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION


This Committee met and the following recommendations were approved:

1. It is recommended that satisfactory facilities be erected on the Canadian border so that livestock imported from Canada may be properly inspected. Among other things these facilities should include shelter from inclement weather, inspection chutes, satisfactory pens, cleaning and disinfection equipment, etc. The facilities should compare favorably with those provided at public stockyards and specifically approved livestock markets. If these facilities cannot be furnished by private ownership, then it is recommended that Congress appropriate funds for this purpose.

2. It is recommended that a study be made of the reported diversions of Canadian livestock released at the border destined to points in the United States but which fail to arrive at destination. It is urged that the state livestock official of the state of destination promptly advise the Animal Inspection and Quarantine Division of the Agricultural Research Service of the nonreceipt of the livestock so that such shipments may be traced and a recurrence of the incident prevented.

3. In order that the livestock industry may be furnished with the best possible service it is recommended that the system of issuing state permits for the admission of livestock be reviewed in an effort to (1) furnish the permit promptly upon receipt of a request and (2) limit the wording of the telegram authorizing the shipment to the fewest possible number of words in order to reduce its cost.

4. Since statistics show that immunization of swine has been on the decrease it is the recommendation of this Committee that every effort be made to publicize the need for vaccination of swine in order to protect the stockyards and transportation companies from losses and interference with operations due to death of animals and exposure of the stockyard facilities and vehicles.

5. As the apparent increase in the incidence of contagious ecthyma (sore mouth) in sheep has caused quite a problem in some public stockyards and to transportation agencies it is recommended that an extensive investigation be made of this disease from an epidemiological standpoint in an attempt to develop a safe and effective vaccine, possibly a Modified Live Virus vaccine.
THEOBALD SMITH (1859-1934) A CENTENNIAL APPRAISAL

MORRIS C. LEIKIND, M.Sc. *, †

Mr. Chairman, Members of the United States Livestock Sanitary Association and Distinguished Guests:

I am greatly honored by the privilege of addressing you on the subject of Theobald Smith and his contributions to Veterinary Medicine and to the livestock industry. Theobald Smith has long been a legend and it may come as a surprise to some that this year we celebrate the one hundredth anniversary of his birth. At the same time we recall with sadness that he died just about 25 years ago. There are persons in this room today who knew him during his lifetime.

Of him an English colleague wrote in 1934:

"He was admittedly the greatest bacteriologist produced by the United States of America. For fifty years his work was continuous and meritorious. His numerous discoveries, many of them of a fundamental nature, entitle him to rank alongside the greatest masters of bacteriology. He was the contemporary of many of the founders of the science and indeed may be regarded as the Last of the Mohicans. It is indeed certain that America in days to come will look on him with that veneration with which France cherishes the name of Pasteur and Germany that of Robert Koch."

I find no reason today to alter the judgment of Dr. William Bulloch, himself distinguished as a bacteriologist and historian of the subject. Legendary as Smith was in his lifetime, his stature grows with the passage of time.

Theobald Smith was born on July 31, 1859 in Albany, New York. His parents were Germans who came to this country shortly after the revolution in 1848 when so many of liberal mind left their fatherland for a freer environment.

He received his early education in the public schools of Albany which in his time must have been very good. He was encouraged to intellectual activity at home and very early acquired those habits of industry and sustained effort which characterized him as a scientist. From his mother he acquired not only a taste for music but also a technical proficiency in the art which enabled him to pay part of his way through college by playing the organ in the school chapel.

Smith graduated from high school at the age of 18 and in a competitive examination won a state scholarship to Cornell. There he quickly distinguished himself by his uniform brilliance in every subject. He was interested * Executive Secretary of the Neurology Postgraduate Training Committee, National Institute of Neurological Diseases and Blindness.
† Formerly, Medical Historian and Archivist, Armed Forces Institute of Pathology, Washington, D. C.
in everything he studied and each of his professors tried to induce him to specialize in their own subject. Smith at first thought of becoming a teacher upon graduation but when he failed to get a suitable job, he decided to study medicine at the Albany Medical College. It is said that the mathematics professors at Cornell long held a grudge against the biologists for having, as they said, ruined a perfectly good mathematician. But the biologists never had any reason to regret their sponsorship of Smith. During the two years required to get his medical degree, Smith managed to spend a semester at the new Johns Hopkins University under Newell Martin and W. K. Brooks. At Cornell he had been taught by such men as Gage and Wilder so we can see he was fortunate in his masters. Upon graduation in 1883 he realized that his two years of study in medical school had not equipped him for practice. Since an apprenticeship under a country doctor did not appeal to him, he decided to return to Cornell for graduate studies in histology. Just about this time Dr. Daniel E. Salmon, Chief of the Bureau of Animal Industry in the United States Department of Agriculture (himself a graduate of Cornell), wrote to Professor Simon H. Gage asking him to recommend a young man to do scientific research in his laboratory. Gage without hesitation suggested the name of Theobald Smith who was already showing promise as an investigator.

Thus in 1884 at the age of 25, Theobald Smith came to Washington to become the Director of the Pathological Laboratory of the Bureau of Animal Industry. He arrived at an exciting and opportune time. Science, especially the biological and medical sciences was in a turmoil. They were in fact being revolutionized by a whole series of new discoveries which were opening vast new horizons. The theory of evolution announced by Darwin and Wallace in 1858/59 was becoming firmly established. The evolutionary concept of an endless chain of being had enormous implications both for veterinary as well as human medicine. Smith was a contemporary of Pasteur, Koch, Lister, Ehrlich, Welch and all of the others who through their creative efforts in establishing the science of microbiology were revolutionizing medical thought and practice. Smith wanted to go abroad and study bacteriology with Pasteur and Koch but could not afford it. So he did the next best thing—he taught himself. Fortunately his early education had been sound for he knew German and French almost as well as he knew English and he read the masters in the original. Gifted with dexterity and manipulative skill he very quickly mastered the basic techniques and was soon making discoveries on his own.

When Smith began his studies there were many fundamental questions still to be answered. For example—in 1883 it was still legitimate to ask—are all diseases of animals and plants due to minute living organisms? Is there a different and specific germ for each disease? Does each variety of germ produce the same disease in all forms of life? How are disease producing forms transferred from one host to another? Why are some stricken with a given disease while their neighbors escape? Why, after recovery from some disease is one safe from future attacks by the same disease?
These were some of the general problems to which Smith and his colleagues in the Bureau of Animal Industry addressed themselves. Theirs was a difficult task not only because they were working in strange and unexplored fields but because, in addition they had to prove themselves. The Bureau of Animal Industry was new and in fact had just been established in the year that Smith came to work there. It had been created “to prevent the exportation of diseased cattle, and to provide means for the suppression and extirpation of pleuro-pneumonia and other contagious diseases among domestic animals.” Pleuro-pneumonia had been introduced into this country in 1843 when a Peter Dunn, a New York milkman bought a cow from the Captain of a British ship at what he thought was a bargain price. Like the famous cow of Mrs. O’Leary, it was a very expensive cow—because it cost the livestock owners millions of dollars. Nevertheless, the legislation creating the Bureau was bitterly opposed by states righters who argued that it was unconstitutional for the federal government to give special assistance to the livestock industry. It was called the “horse doctor bill” and Congress was petitioned not to saddle still more Washington Bureaucrats on the overburdened and long-suffering taxpayers. Furthermore, the opponents argued—the disease was a myth anyway and besides—state regulations were adequate to control its spread. Happily the Bureau was established and it may be mentioned here that after five years of work at a cost of about a million and a half dollars, pleuro-pneumonia was eradicated from this country.

The Director of the Bureau, Doctor Salmon, whose name is perpetuated in the Salmonella group of organisms, was closely associated with Smith for 11 years, and they jointly published many important reports and bulletins. From 1886 to 1895, Smith also held the post of Professor of Bacteriology at the Columbian College, now the George Washington University.

In 1895 began another phase of his career. He went to Boston as head of the Pathological Laboratory of the Massachusetts State Board of Health and in the following year was appointed to the Chair of Comparative Pathology at Harvard. Twenty years later, in 1915 he left Boston to go to Princeton as Director of the newly established Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research. In 1929 he retired with the title of Director Emeritus. His intellectual vigor undiminished he remained active until the very end of his life on December 10, 1934.

Now let us review briefly some of his accomplishments. All who knew him are agreed that he was a prodigious worker. One of Smith’s junior assistants was once queried about his Chief’s working habits. The reply was, “Well, Dr. Smith occasionally takes a Sunday afternoon off.” His bibliography includes 280 publications or an average of 5.3 papers per year for 50 years. Some scientists are single shot rifles—Smith was a machine gun with the remarkable ability to place his shots in the bull’s eye or very close to it. To make his contributions a bit more significant let us analyze the figure of 280 a bit more closely. From 1883 to 1932 he wrote 90 papers which may be classified as in the field of general bacteriology and pathology. During the same period, 1884-1933 to be exact he produced 39 papers on tuberculosis. The subjects of swine plague and hog cholera which occupied some of his time
from 1885-1889 resulted in 22 papers. On the subject of sanitation, 21 papers appeared from 1886-1927. Immunology was enriched by 43 papers from 1886-1934. On Texas Fever, the disease with which his name is forever associated he wrote 12 papers during the period of 10 years from 1889-1899. These included the classic Bulletin No. 1 of the Bureau of Animal Industry. To Parasitology he contributed 22 papers from 1889-1927. And lastly, as his wisdom increased, his writings took a philosophical turn culminating in his classic book Parasitism and Disease. These meditative papers, mostly addresses and essays numbered 30, from 1900-1934.

There is not time to discuss in detail all of Smith's contributions and therefore during my remaining time I wish to analyze briefly a few of his more significant studies.

One of his earliest papers was on the isolation and cultivation of the tubercle bacillus which has been discovered by Robert Koch in 1882. Smith's work on this subject led him in two directions. As he studied not only the tubercle bacillus but a number of other organisms including the hog cholera bacillus and others, he recognized very early the variability of pathogenic organisms. This was long before the days when the distinctions were made between rough and smooth colonies and the serological studies which were associated with them. Nevertheless, Smith was one of the pioneers in the study of what later became known as the phenomenon of bacterial dissociation. Thus as early as 1903 he showed that the flagella of motile organisms may have antigenic constituents different and separable from those of the somatoplasm of the organism a concept much later developed in its theoretical and practical aspects by Felix and his followers.

The other path down which his early studies on tuberculosis led him was his differentiation of human and bovine strains. For some years after Koch's discovery of the tubercle bacillus it was assumed that all mammalian tubercle bacilli were identical and that the bovine disease was interchangeable with the human. Koch himself believed this and indeed it was a generally accepted opinion till 1896. In that year Smith re-opened a subject everyone thought was closed. In a brilliant series of papers he showed that bovine strains of the tubercle bacillus were far more virulent for rabbits, guinea pigs and cattle, than were human strains. In 1904-05 he demonstrated a subtle biochemical difference between types in their reaction to phenolphthalein in glycerine broth. Almost at the same time that he began his work on tuberculosis Smith, together with his Chief, Salmon, began to study several other animal diseases, the etiology of which was then unknown. One of the most important epizootics was the so-called swine plague. It was found that actually two diseases were involved from one of which the hog cholera bacillus was frequently isolated, the other in which a pasteurella-like organism B. Suisepticus was predominant. Although Smith's work was too early to have gotten on the train of the virus agent—discovered in 1903 by de Schweinitz and Dorset, he and Salmon did come up with something equally fundamental. For they demonstrated in 1886 that it was possible to prepare protective vaccines against certain bacterial diseases by using killed cultures as antigens. This was one of the most important discoveries in the then new
The science of immunology and led ultimately to the development of such vaccines as those against cholera, typhoid and plague. An incidental discovery was experimental scurvy which could have ushered in the vitamin era much earlier.

We come now to what some regard as Smith's greatest piece of work—that on Texas Fever. This was a disease which had been prevalent in the United States from colonial times. However, it did not become a real problem until the country began to expand and railroads began to spread their network of steel ribbons over the land.

As with all infectious diseases before the germ theory of disease was accepted there were all sorts of guesses about the cause but no hard facts. Even those observations which later turned out to be true were first scoffed at.

When Smith in association with his colleague Kilbourne first began to work on the disease, a few facts were known. Thus Salmon had already outlined the geographical limits of the disease as roughly between the 37th and 38th parallels north latitude. Healthy cattle going below this line got the disease while southern cattle coming up north of the line carried the disease with them. Farmers had long suggested that a cattle tick was in some way associated with the disease. When Smith began his work, the tick theory was put to test, at first, not to prove that the guess was true but simply to eliminate it from the guessing, to clear out the underbrush as it were. But it was quickly found that the tick was indeed the key to the problem. The relationship of insects to the transmissions was still obscure.

As early as 1879 Sir Patrick Manson a physician in China had shown that mosquitoes could transmit the agent of filariasis, a parasitic worm. But his demonstration was incomplete and it was regarded as an isolated case, exerting little influence on epidemiology, theoretical or applied. In 1889 in association with Kilbourne, Smith began a beautifully planned and executed series of experiments which not only solved the mystery of Texas Fever but opened up a new era in epidemiological thinking and in the control of many communicable diseases. We know that Smith had already begun to think about the problem of Texas Fever as early as 1884. By the time he began his famous experiments a bacterial cause had been ruled out.

Without going into detail, the following major results were demonstrated:

(a) Northern cattle placed in the same field with recently imported southern cattle, contracted the disease in a few weeks.

(b) If the ticks on southern cattle were removed before they were penned up with northern animals no disease resulted.

(c) If northern cattle were fed on a field infested with ticks from southern cattle the disease appeared.

To further reinforce the case, Texas Fever was produced by the bites of young ticks reared in a laboratory from eggs laid by parent ticks which had lived on southern infected cattle. The disease was also produced in the north
THEOBALD SMITH

During the winter in a heated stable by the bites of viable ticks. Finally, the causative organism, a protozoan parasite was demonstrated in the blood. During the course of the experiments Smith wrote in a letter:

"After it had been shown that the disease failed without ticks, everything was still to be done; what was the cause, the nature of the disease? How did the animals become infected? Did they eat the ticks? Did the young ticks introduce a toxin? Were the intra-globular bodies degenerated blood cells? To say that the protozoan parasite passes from old to young ticks through the eggs and then into their mouth parts and thus to the cattle requires some proof before it would be accepted at that time. It took four years of slavery at the microscope, at autopsy, at watching ticks hatch from the egg, and when placed upon cattle, to see the deadly Texas Fever re-appear."

The conclusions of this notable series of experiments were: Texas Fever is a disease of the blood characterized by the destruction of red blood corpuscles. The destruction of the red cells is due to a micro-parasite living within them. Cattle from permanently infected territory though otherwise healthy carry the parasite of Texas Fever in their blood. Texas Fever in nature is transmitted by the cattle tick. The infection is carried by the progeny of the ticks which matured on infected cattle and is inoculated by them directly into the blood of susceptible cattle."

Here now was the first clear and adequate proof of a protozoan disease of higher mammals transmitted through an intermediate arthropod host. The way was now clear, not only for the control and eradication of this disease but a signpost had been erected indicating the road to be opened in the control of other similar diseases. All this at a cost of $65,000.

In 1894 Bruce of the Royal Army Medical Corps showed that the tsetse fly in Africa was the vector of nagana a trypanosome disease of cattle. A few years later came the demonstration by Ronald Ross of the mosquito transmission of malaria and the similar demonstration by Reed and his colleagues for yellow fever. Typhus fever, dengue fever, bubonic plague and many other diseases were soon added to the list of insect borne plagues.

A discovery, Smith once pointed out, may mark the beginning of an epoch, but can never make it. Smith in his Texas Fever work certainly marked the opening of an epoch. Smith had scarcely finished his work on Texas fever, described by some as yet sceptical veterinarians as “a romance in pathology,” when he was attacking another problem—Blackhead in turkeys. The disease seemed to be especially prevalent in Rhode Island so Smith went up to the experimental station at Kingston to study the matter first hand. He soon was able to demonstrate a protozoan parasite not before recognized as the causative organism. Because of its localization in the intestinal tract and liver it was at first thought that the disease transmitted through the droppings of the birds which were eaten by healthy birds. But it was not so simple. Later investigation showed first of all that the organisms were not really an amoeba but a flagellate \textit{Histomonas meleagris} and that the infectious cycle required the cooperation of a nematode worm. The disease was controlled by eradicating the worm from turkey farms. In 1895 Smith left Washington for Massachusetts. There he did important work on the stand-
The achievements of science are neutral, until applied by man for purposes constructive or destructive, good or evil.

—Theobald Smith
ardization of vaccines and anti-toxins noted incidentally the phenomenon of anaphylaxis, a discovery which he casually passed on to Paul Ehrlich in a letter with the suggestion that he put one of his assistants to work on it. For years it was known as the Theobald Smith phenomenon.

A second major discovery was the fact that in a pregnant guinea pig, immunized against diphtheria, a passive immunity is transmitted to the offspring lasting several months.

Later, when he went to Princeton he followed up this observation with a study on new-born calves. He found there that a passive immunity to colon bacilli only came through with the colostrum. Thus if this first milk were denied to the calves they can be overwhelmed by colon bacilli. But if allowed to take it, passive immunity tides them over until their own body defenses can take over.

I wish there were time to discuss further the work of this great investigator for such he remained all his life. He shunned administrative work and for this reason had refused, early in the century, the Directorship of the Rockefeller Institute which was offered him. At that time, he wrote, “I anticipate much from this new institution and eventually it will be richly endowed, I believe. But, after all, you and I know, that research cannot be forced very much. There is always the danger of too much foliage and too little fruit.”

I mentioned earlier that in addition to his scientific papers he wrote a number of essays which may be classified as philosophical. I want to conclude with one item which, written as a letter, perhaps sums up the man better than anything which may be said about him. It was written shortly before his death to his friend Professor Krumbhaar of the University of Pennsylvania, who has kindly permitted the use of this letter.

October 11, 1933.

DEAR DR. KRUMBHAAR:

As we grow old we come to the end of an individual era in which we have tried “to do our part.” We begin to realize the important function of the past in shaping the future. We also feel the at times benumbing and soporific effects of that past to be gotten rid of.

It is not uncommon for the younger generation to criticise or even disregard earlier work because it is not complete from the more recent standpoint. No research will answer all queries that the future may raise. It is wiser to praise the work for what it has accomplished and then to formulate the problem still to be solved. It is not profitable to enter into controversies especially with those working in another geographic area or continent unless the material on which their researches are based, has been examined.

To those who have the urge to do research and who are prepared to give up most things in life eagerly pursued by the man in the streets, discovery should come as an adventure rather than as the result of a logical process of thought. Sharp, prolonged thinking is necessary that we keep on the chosen road but it does not itself necessarily lead to discovery. The investigator must be ready and on the spot when the light comes from whatever direction.
There are many to compete with the young investigator. Opportunities for research have been increased a hundredfold in the past half century. More and more our colleagues fail to understand our work because of the high specialization of research problems. We must not be discouraged if the products of our labor are not read or even known to exist. The joy of research must be found in doing since every other harvest is uncertain and even the prizes do not always go to the discoveries to which we would assign them. Research has deserted the individual and entered the group. The individual worker finds the problem too large, not too difficult. He must learn to work with others.

In bacteriology and pathology research is slowly receding from the ultra practical point of view of the early leaders. One group thought it possible to catch all bacteria in transit from one victim to another and to suppress disease in this way. Another group thought that a vaccine could be prepared for every disease. We have learned much since then and have become quite humble. Our researches no longer lead to Public Health regulations. They are more elusive and difficult to fit into any scheme for decreasing the incidence of disease. We must be content with the vision of future usefulness.

In general, a fact is worth more than theories in the long run. The theory stimulates but the fact builds. The former in due time is replaced by one better but the fact remains and becomes fertile. The fertility of a discovery is perhaps the surest measure of its survival value. What is one man's meat is another's poison in research as in other vocations. Temperament goes far towards deciding our course. In the three different environments in which I have spent my active life I have always taken up the problems that lay spread out before me in the new environment, chiefly because of the easy accessibility of material without which research cannot go on for in the early years material and resources were exceedingly scant and this meagerness determined the direction and scope of all research. My interest in a problem usually lagged when certain results could be clearly formulated or practically applied. To continue and analyze still further every link of the established chain either failed to hold my interest or was made difficult or impossible for causes lying outside the problem. As I look back it is precisely these links that have provided innumerable problems to others. Each link has grown into a chain and the end of successive chain making is not in sight.

Sincerely yours,

Theobald Smith
Before prognosis and therapy can successfully be applied to a disease, the pathological events taking place during the course of that disease should be understood as fully as possible. Then the outcome of the disease and the effect of various therapeutic measures can be correlated with the pathogenesis.

Anaplasmosis produces rapidly progressive anemia in adult cattle. The blood changes in this disease should be characteristic of any acute anemic condition showing rapid decreases in erythrocytes and hemoglobin followed, if recovery ensues, by changes indicative of increased hematopoiesis. Correlation of these hematological changes with death or recovery and the apparent value of various treatments may provide improved prognosis and therapy in anaplasmosis.

While it is known that the tetracycline drugs will halt the increase in anaplasma infected erythrocytes (1, 2) they are not beneficial when used during or later than the maximal anemia (3). When these drugs are used early in the development of the anemia they are helpful in preventing death (3). Study of the blood changes during this disease may provide reliable signs indicating when tetracycline therapy will be most useful.

There is considerable controversy concerning the treatment of anaplasmosis during maximal anemia. Opinion among practitioners in enzootic areas appears to be quite evenly divided between those that feel treatment is beneficial at this time and those that feel the excitement and exertion incident to treatment is more likely to cause death than to help the animal. Much of this controversy is related to whether or not the animal will recover without treatment. Certainly the ability to make a prognosis of recovery without treatment could prevent the unnecessary over-treatment of many cattle. Blood changes indicating the amount of increased hematopoiesis should indicate recovery from the anemia.

The hematological changes occurring during the course of anaplasmosis were studied in detail for the past few years. The data from 16 adult cattle presented here are representative of the typical blood changes in anaplasmosis.

**METHODS**

Twenty adult cattle were given anaplasma infected blood from the same carrier cow. Ten of the cattle were two-year old Hereford steers. The other
10 cattle were mixed dairy type cows ranging in age from three to eight years.

Three of the dairy type cattle and one of the Hereford steers died. The data from these cattle were eliminated from this study because the interest here is in the disease from incubation through convalescence.

RESULTS

Figure 1. Hematological values during acute anaplasmosis.

Figure 1 graphically represents the means of the red blood cell counts, hemoglobin, hematocrit and percentage of red blood cells containing anaplasma bodies found in the 16 cattle in this study. These values are presented in order to provide readily recognized references to the usual course of anaplasmosis.

The curve of the red blood cell count shows the typical slope in which approximately 1,000,000 red blood cells are lost daily during the period of greatest decline reaching minimum values on the tenth day followed by a very gradual increase toward normal. Hemoglobin and hematocrit values tend to follow the red blood cell changes.

The curve of infected red blood cells is, as usual, quite symmetrical reaching a peak on the seventh day, and, after dropping rapidly from the peak, tails out to the sixteenth day.

The clinical condition of these cattle may be inferred from this figure. The values shown by the red blood cell, hemoglobin and hematocrit curves indicate that these cattle became clinically ill between the fourth and sixth days.

From the sixth day through the tenth day the cattle became progressively more anemic and developed the typical signs of anaplasmosis. From the tenth to the thirteenth day is the period of maximum anemia, but during this period, the cattle were showing clinical signs of recovery, such as the desire to drink water and the relief of constipation. The period when the cattle were most likely to die was from the eighth through the tenth days. Of the four cattle which died, two died on the eighth day and one each on the ninth and tenth days.
Since the signs of anaplasmosis and the causes of death are apparently due largely to anemia, study of the values showing the type of anemia and the response of the hematopoietic system to the anemia should provide a rational approach to understanding the disease.

Some evidence is presented in figure 2 that the anemia in anaplasmosis is due to destruction of the infected red blood cells. This figure again shows the mean values of the percentage of infected red blood cells found in this study. In addition the figure also presents the mean values of the amount of urobilinogen in the feces of these cattle. Since urobilinogen results from the breakdown of the protoporphyrin portion of hemoglobin and is excreted by the liver in bile, the urobilinogen found in feces is generally proportionate to the destruction of red blood cells. Due to the time necessary for catabolism and passage through the intestinal tract the rise in urobilinogen appears to follow the drop in red blood cells by approximately two days.

One must realize that factors other than destruction of mature red blood cells contribute to the amount of urobilinogen found in the feces. Therefore, the fecal urobilinogen cannot be used to accurately measure the amount of red cell destruction. In the event, however, of marked anemia accompanied by extreme increases in fecal urobilinogen the other factors usually are not significant.

As presented by figure 2 the mean values of fecal urobilinogen prior to anemia were less than one mg. per 100 g. of feces. By the third day there was a slight rise to one mg. of urobilinogen followed by an increasingly rapid rise to 61 mg. by the eleventh day. The decrease in the urobilinogen after the eleventh day was equally as rapid as the earlier increase and dropped below one mg. per 100 g. of feces on the eighteenth day.

The rapid rise in urobilinogen, following as it does the rapid decrease in red blood cells, is indicative of an hemolytic anemia. The similarity of the urobilinogen curve with the curve of the infected red blood cells, both in time and slope of the curves, may be additional evidence that it is the infected red cells which are destroyed.
Figures 3 and 4 represent values determined from the circulating blood that give some indication of what is happening in the hematopoietic system. Figure 3 contains the values which may be calculated from the hematocrit, hemoglobin and red blood cell count. These values are the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) which measure respectively the size of the red cell, the amount of hemoglobin the red cell contains, and the percentage of the cell volume occupied by hemoglobin. Immature red cells are larger than adult cells; they contain a greater amount of hemoglobin, but because of their increased size, the hemoglobin occupies a smaller percentage of their volume. Therefore, an increase in either MCV or MCH or a decrease in MCHC is indicative of immature red blood cells in the circulating blood and, by inference, increased erythropoiesis.

The measurements presented in figure 3 show a beginning increase in erythropoiesis on the seventh day as indicated by a slight increase in MCV and a slight decrease in MCHC. The corresponding increase in MCH is delayed until the eleventh day. Erythropoiesis continues to increase during
most of the remainder of the period studied as shown by the increasing size 
and hemoglobin content of the red blood cells.

The fact of increased erythropoiesis during the latter part of acute ana-
plasmosis is further confirmed by the information in figure 4. The bovine 
normally shows no reticulocytes in the circulating blood. The presence of 
any reticulocytes in the peripheral blood, therefore, is indicative of increased 
erythropoiesis.

Reticulocytes are shown in figure 4 to appear on the sixth day, to increase 
in percentage of total red blood cells until the fourteenth day when they 
reached 25 percent. The percentage of reticulocytes then decreased to 2.5 
percent on the twentieth day.

Stimulation of the bone marrow by anemia is apparently confined not only 
to increased erythropoiesis but also to increased granulopoiesis. This phe-
omenon is shown by the similarity of the curve representing the white 
cell count of circulating blood to the other curves indicating increased 
erythropoiesis. The number of leukocytes increased from the normal of 
8,000 per cmm. of blood on the seventh day to approximately 19,000 per 
cmm. of blood on the fifteenth day followed by a decrease to almost normal 
on the twentieth day.

**DISCUSSION**

These data may be used to divide the course of anaplasmosis into various 
stages. Classifying the course of the disease and understanding, at least in 
part, the changes that are taking place in the anemia during each stage pro-
vides a logical basis on which to treat the animal and to form a prognosis.

The period from entry of the organism to the appearance of one percent 
anaplasma infected red blood cells is called the incubation stage. The time 
from the appearance of one percent infected red blood cells to the appearance 
of signs of increased hematopoiesis may be termed the developmental stage 
since it is during this period that the anemia develops. The time from the 
appearance of increased hematopoiesis to the return of the blood values to 
normal may be called the convalescent stage. The term “convalescent” is 
used here in a restricted sense of referring to laboratory signs of recovery 
from the anemia rather than in the broader sense of clinical recovery from 
sickness. While, in our experience, the two events usually coincide, we have 
had animals which died as many as two days subsequent to the appearance 
of reticulocytes. The carrier stage extends from the end of canvalescence 
to the end of the animal’s life in most cases.

For the treatment and prognosis of the animal acutely ill with anaplasmosis, 
the developmental and convalescent stages are of greater importance. The 
line between these two stages is not sharply defined by all of the measurements 
which show increased hematopoiesis presented in this study. For example, 
reticulocytes appear on the sixth day, increased MCV on the eighth day, 
decreased MCHC on the ninth day, and increased MCH on the eleventh day. 
This wide spread in the appearance of these blood changes is caused by the 
method of presenting the data. The results from any one animal show the 
appearance of reticulocytes followed by changes in the other values within
two days. By combining the data from a number of animals into one figure, however, the first appearance of reticulocytes in any animal is recorded on figure 4 while it may be some time before the mean values of the other measurements show significant changes.

The tetracycline drugs are most widely used for the treatment of anaplasmosis because of their ability to halt the increase in the number of infected red blood cells, yet they frequently fail to prevent death. This study presents some reasons for their failure and some basis for estimating when these drugs can most effectively be used.

The data in figure 2 suggest that the anaplasma infected red cells are those cells which are destroyed. Treatment, then, with the tetracyclines should reduce the anemia by stopping the increase in infected cells. As can be seen in figure 1, the time at which the treatment is given is of extreme importance since the number of infected cells decreases rapidly without treatment after the seventh day. In order to appreciably reduce the number of infected cells this treatment must be used by the fifth day. Certainly after the sixth day such treatment cannot be expected to change the course of the disease. Since the clinical signs of anaplasmosis only begin to appear between the fourth and sixth days the cattleman must be exceptionally observant to recognize the disease early enough for the tetracycline drugs to be of value.

Since the appearance of reticulocytes approximates the time of maximum percentage of red cell infection, their appearance may be taken as the most easily determined point dividing the time when tetracycline treatment may be effective from when it may be of little or no value. Since the appearance of reticulocytes is one of the earliest signs of convalescence it may be generally stated that the tetracycline drugs are effective only if given during the developmental stage of the disease.

The hematinic drugs theoretically may have some value when used during the developmental stage. This is particularly true of iron, cobalt and vitamin B₁₂ when anaplasmosis is complicated with parasitism or malnutrition.

Most cases of anaplasmosis are first seen early in the convalescent stage. The first two or three days of this period are most critical because there is maximum anemia with only slightly increased erythropoiesis. When used at this time, the value of the treatments currently available should be carefully judged against chances for recovery without treatment.

The hematinic compounds probably do not have time to further stimulate erythropoiesis sufficiently to influence the mortality rate. Blood transfusion is the logical treatment at this critical time but certain problems in its use destroy much of its value. Simple calculation shows that transfusing less than one gallon does little to increase the red cell mass. Under most circumstances in practice the blood must be given rapidly. The sudden increase of blood volume by the transfusion of one gallon of blood frequently causes cardiac embarrassment in a heart already damaged by anoxia.

The effectiveness of treatment during this period of maximum anemia must be compared with the chances for recovery without treatment. The exercise and excitement incident to treatment may easily cause death from anoxia. Figures 3 and 4 contain evidence that increasing numbers of
erythrocytes are entering the blood at this stage indicating that the animal is already recovering from the anemia. Consequently, there is the constant risk in treating cattle at this time that one may kill an otherwise recovering animal.

After the critical first two or three days of the convalescent stage the percentage of reticulocytes, the MCV and the MCH increase appreciably. Normoblasts, basophilic stippled erythrocytes and polychromasia then appear on the blood smears. Many cases of anaplasmosis are first seen at this time. Blood transfusions and hematinic drugs may be given during this period to hasten complete recovery without fear of causing death.

SUMMARY

Anaplasmosis may be divided into four stages: incubation, developmental, convalescent and carrier on the basis of changes in the circulating blood. Cases of acute anaplasmosis are seen in the developmental and convalescent stages. The developmental stage is characterized by increasing numbers of anaplasma infected erythrocytes and progressive normocytic normochromic anemia. The convalescent stage is characterized by decreasing numbers of infected erythrocytes and macrocytic anemia with all the changes indicative of stimulated hematopoiesis.

The tetracycline drugs appear to be effective in acute anaplasmosis only when used in the developmental stage. In order to be of any value in decreasing the mortality the hematinic drugs should be used during the developmental stage. The benefits derived from any treatment, including the present methods of blood transfusion, used during the convalescent stage must be judged against the dangers of excitement, exertion, damage by the treatment itself and against the fact that signs of recovery are already present.

BIBLIOGRAPHY

FIELD CONTROL STUDIES ON ANAPLASMOSIS

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T. O. ROBY, D.V.M., M.S.,* and W. H. MARTIN, PH.D.†

Losses from bovine anaplasmosis have been particularly heavy in many herds of cattle in enzootic anaplasmosis areas of the Southeastern United States during the last few years. Apparently, the conditions favorable to production of large numbers of cattle in these areas are also favorable to transmission of the disease. Outbreaks of the disease beginning in the early summer and continuing until fall have been common in the Mississippi River Valley and along the coastal areas of the Gulf of Mexico. Large biting insect populations and the presence of anaplasmosis-infected carrier cattle mixed at random with susceptible animals are believed to be the chief predisposing factors causing outbreaks of the disease in this region.

The anaplasmosis syndrome is much the same wherever the disease occurs. The natural vectors, however, believed to be chiefly responsible for transmission of anaplasmosis are very different in the Southeastern United States from those in the Rocky Mountain and West Coast regions. In the Rocky Mountain and West Coast regions the natural vectors of primary importance are believed to be the Rocky Mountain wood tick, Dermacentor andersoni, and the Pacific Coast tick, D. occidentalis. Biting insect vectors in this area are considered to be of secondary importance to transmission. In the Southeastern United States biting insects are considered to be the natural vectors of primary importance. These insects are very abundant in the enzootic anaplasmosis regions. The two major ticks of cattle in the Southeastern region are the Lone Star tick, Amblyomma americanum and the American dog tick, D. variabilis (1). The Lone Star tick has not been experimentally incriminated in anaplasmosis transmission (2). The American dog tick is capable of transmission, but only from stage to stage. The immature stages of this tick are rarely found on cattle and transovarian transfer of the disease agent has not been demonstrated. Further, anaplasmosis is common in some areas of the Southeastern region where ticks are not a common ectoparasite of cattle. In this region natural wild animal reservoirs have not been implicated as have the deer in California (3).

Until the last few years no definite progress had been made on practical means for combating bovine anaplasmosis. Veterinarians used a variety of nonspecific drugs for treating clinical cases and obtained variable results. The discovery that several broad spectrum antibiotics have inhibitory action on the causative organism, Anaplasma marginale, (4) has made it possible to treat the disease with greater confidence. The development of oral feeding

* National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, Beltsville, Maryland.
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of antibiotics for both prevention and elimination of infection has been shown to be practical under some circumstances (5, 6). The detection of carrier cases of anaplasmosis by the complement-fixation (c.f.) test (7) has made incidence surveys and experimental field trials possible. Various combinations of control methods utilizing segregation or isolation of carriers, removal of carriers for slaughter, or treatment of carriers have been employed successfully in limited trials (6, 8, 9 and 10). These methods of control have not been thoroughly tried in many Southeastern areas where anaplasmosis is a major problem.

The only cattle which are not susceptible to the disease are those which have become infected and lived to remain carriers. In the enzootic areas many cattle are carriers and people are often reluctant to attempt to establish herds free from anaplasmosis. However, in order to gain more information on methods and procedures for controlling the disease, sound experimental field trials are necessary. Such trials to study the effect of available control methods can and should be made.

This report summarizes results that have been obtained to date where anaplasmosis field trials are being conducted. These trials are being done cooperatively between the states and the Agricultural Research Service in herds in several enzootic areas of the Southeastern United States.

Our present scientific knowledge of anaplasmosis indicates that control of natural mechanical transmission is the key to the solution for helping the southern cattlemen prevent anaplasmosis losses. Disease transmission control depends on three principal factors; (a) The infectivity level of the blood of carrier cattle, (b) the insect vector population densities for any given area, and (c) the lapse of time between insect feedings on infected and susceptible cattle. Control of any or all of these factors has an immediately favorable effect on reducing the disease transmission rate. Most experimental field trials have been designed to evaluate methods of controlling these factors.

Assistance for controlling bovine anaplasmosis is usually requested at the time an outbreak of the disease is occurring. When the acute or clinical stage of the disease develops in a herd, it is often found that many susceptible cattle have become infected without showing severe clinical symptoms. Variations in the degree of anaplasmosis infection extend from the sub-clinical or inapparent type to the acute and peracute types. Testing has shown that considerable anaplasmosis may be found in some herds in which the disease has not been considered a widespread infection or even recognized. The following two herds are presented as examples to illustrate the insidious nature of bovine anaplasmosis and to show how well masked the presence and spread of infection can be:

The first herd was a crossbred beef herd located in Louisiana. Clinical diagnosis of anaplasmosis had not been reported although the herd was in an enzootic area. The herd tests in the spring of 1958 showed the majority of anaplasmosis c.f. reactors were cows, three years of age or older. Serums from 32 percent of 240 cows showed a positive c.f. reaction. A control program at this time was not feasible. The herd was retested that winter to
determine how much transmission had taken place during the insect vector season. The reactor incidence of the same group of cows had increased to 67 percent. Several acute cases of anaplasmosis have been reported in this herd during the insect vector season this year.

The second herd was a beef herd located in Missouri. In the fall of 1955 a test on the herd of 550 animals revealed a reactor incidence of 18 percent. Three years later—the same basic herd was tested again and now the reactor incidence was 46 percent of 575 animals. Clinical anaplasmosis had occasionally been diagnosed in the herd. No program of control had been followed during the three-year period between the two tests. These examples are given to show the extent to which anaplasmosis can progress when the disease is allowed to spread unchecked under natural conditions. The number of infected animals in a herd is not necessarily an indication of the degree of losses sustained.

Cooperative field trials on anaplasmosis control have been conducted in a group of privately owned herds in western Tennessee. The disease is a serious problem in many herds of the area which lies along the Mississippi River. Following an initial herd test, the owners have chosen either to sell their c.f. reactors for slaughter or to keep them in separate pastures away from the c.f. nonreactors. In most instances acute cases of anaplasmosis have not appeared after the serological reactors had been separated or removed from the herds.

It has been difficult to maintain continued interest and cooperation in further retesting of some herds under these circumstances. Where a large number of reactors are revealed on the initial herd test, the owner usually holds them until conditions are favorable for marketing. One purebred herd owner was exceptionally interested and serious about eradicating anaplasmosis from his herd. On the first herd test in the fall of 1957 104 reactors and 11 suspects were found in the herd of 266 animals. Sixty cows had died from anaplasmosis during the summer of that year. The owner elected to dispose of his reactors and suspects by immediate slaughter. Two years later in August of 1959, no reactors and two suspects were found on the fourth retest of this herd. There have been no symptoms of the disease for over a year. These results were attained even though the herd was adjacent to an infected herd. The purebred herd is pastured along a river bottom where mosquitoes and horse flies are abundant.

A more concerted educational effort is now being initiated in Tennessee to coordinate the anaplasmosis field trial studies with the activities of the herd owners and the practicing veterinarians who do the bleeding. The purpose is not to increase the number of the field trial herds but to gain more information and data on the best methods for controlling the disease in that area. During the last three years, 28 herds have been initially tested; two have been sold out completely; and testing in 10 has been discontinued for lack of cooperation. Of the 16 remaining herds, 13 are on a test-and-slaughter program, and three are on a segregation program.

In Western Mississippi extensive losses from bovine anaplasmosis occurred in the Mississippi-Yazoo Delta region in 1957 and 1958. This area has
FIELD CONTROL STUDIES ON ANAPLASMOSIS

many large beef herds in an enterprise secondary to cotton farming. The biting insect populations become very large from spring to fall. It was estimated that the economic death losses from anaplasmosis in Mississippi, which occurred principally in this Delta region, were approximately three million dollars in the 1958 season. Cattlemen of the area were very much concerned about anaplasmosis and were receptive to any suggestions which might help decrease the losses from the disease.

Discussions with the Mississippi Delta Council, the Mississippi State Veterinarian, and the Agricultural Research Service led to agreements for anaplasmosis control field trials in the area early in 1959. It was originally intended to conduct the work on all herds within a limited area of the Delta region. However, interested owners from all sections of the Delta region requested participation in the trials, and the program was enlarged accordingly.

The general program was to conduct two tests prior to the insect season. Negative animals revealed on the initial test were retested 30-45 days later. The negative animals were pastured throughout the insect season in areas separate from the reactors. The expense of blood collection and subsequent identification of reactors was borne by the owner. A third test on the negative group was to be made in the early winter after the insect season. The purpose of the third test was to measure the amount of transmission occurring in the negative groups.

The initial herd tests were begun in February and completed in March. A total of 79 herds containing 14,994 cattle in 16 counties were tested. The average incidence of c.f. reactors and suspects was slightly more than 50 percent of the total number tested. It was found that individual herd incidence patterns varied from no reactors in a few herds to almost complete reactors in several herds. The retesting of the negative animals, done between 30 and 45 days following the initial herd test, showed 3.4 percent new reactors. The program was late getting started and as a consequence it was not possible to conduct all retests and separation of reactors from all the herds before the insect season began. Insect control programs were conducted at the owner's discretion and were carried out in 1959 in a similar manner to previous years.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Anaplasmosis Control: Summary of 1959 Field Trials in the Mississippi-Yazoo Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Incidence (Percent)</td>
<td>Herds Tested (No.)</td>
</tr>
<tr>
<td>0- 25</td>
<td>28</td>
</tr>
<tr>
<td>26- 50</td>
<td>16</td>
</tr>
<tr>
<td>51- 75</td>
<td>18</td>
</tr>
<tr>
<td>76-100</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
</tr>
</tbody>
</table>

* Estimated losses during season prior to field trial.
† Estimated losses during season of control field trial.
The majority of the 79 herds followed a program of segregation. There were some variations in the control procedures, however. In eight high incidence herds, the owners chose to remove the negative animals and kept a reactor herd. In several low incidence herds the owners elected to dispose of their reactors for immediate slaughter. In a few herds the reactors were treated with antibiotics and kept in the herd.

The over-all results are encouraging from the standpoint of reduction of losses and new transmission. The extent of losses this year was low compared to those which had occurred during the previous year in the same herds (Table 1). There were, however, 19 herds in which clinical cases of the disease were reported during the 1959 anaplasmosis season. In many of these herds the reason for further transmission was apparently due to the delay in separation of reactors and negatives after insects had become numerous. There were several herd owners who did not segregate or follow a definite control program after the initial test. Death losses and clinical cases were extensive in these nonsegregated herds which indicated that conditions for natural spread of the disease were present in the area.

At the present time, the c.f. negative groups that were established last spring are being retested to determine the number of reactors occurring after the insect season. To date, the third test in 27 herds has shown 10.8 percent new reactors out of a total of 2673 previously negative animals which were segregated from other reactors in the spring. In cooperation with the Delta Branch Experiment Station, several herds in the area are being intensively studied from the standpoint of various insecticide and antibiotic treatments in relation to their effect on controlling disease transmission. Preliminary findings indicate that both regular use of insecticides and antibiotic feeding can provide considerable support to the segregation program. The field trial studies will be continued until more detailed recommendations on the control of the disease in this area can be made.

These limited anaplasmosis field trials lead us to conclude that c.f. testing and segregation of reactors from non-reactors have greatly reduced the probability of losses and infection in enzootic areas of the Southeastern United States. Further trials utilizing testing, segregation, vector control and antibiotic feeding should be made.

ACKNOWLEDGMENTS

The authors express appreciation to Dr. C. E. Kord and Dr. V. D. Chadwick, the State Veterinarians of Tennessee and Mississippi, respectively, for their interest and support of the work described in this report. Also, acknowledgment is made to the many interested and cooperative persons in Mississippi who made the work there possible.
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REPORT OF THE COMMITTEE ON ANAPLASMOSIS


Losses from anaplasmosis continue to be a major problem to the livestock industry. There has been no appreciable decrease in losses the past year.

Last year the Committee made the observation that additional information on anaplasmosis is gradually becoming known. It is more and more evident that the disease problem varies in terms of incidence, methods of transmission and reservoirs in the different geographical regions of the country. For these reasons the Committee recommended emphasis be continued on fundamental research and field trial control programs to broaden the understanding of regional variations. A speaker has told of progress along these lines.

Dr. E. H. Willers, the official veterinarian for Hawaii, reports on the anaplasmosis program in that state as follows:

“For the second consecutive fiscal period, testing for anaplasmosis in Hawaii during 1958-1959 failed to uncover any carrier animals other than recent imports. If the same results are obtained throughout the ensuing fiscal period it is probable that the program will be reduced to testing of imported cattle only.

“Six reactors were found among 2,155 animals imported, of which five were detected on entry test and one was found on a 60-day retest following a negative entry test. To determine whether or not animals showing positive reactions to the complement-fixation test were true carriers of anaplasmosis, 15 calf trials were conducted during the fiscal year. Only one test calf developed symptoms of the disease. This calf was the only one of the series that was inoculated with whole blood from an imported animal. The 14 other calves were inoculated with whole blood from 4+ reactors found among native cattle. All 14 trials gave negative results, and the test calves were found susceptible to challenge with blood from a known carrier maintained in isolation for this purpose.

“Three valuable Charbray bulls which arrived in March, 1959 were returned to the ranch of origin. These bulls originated among a group of animals, a few of which gave positive reactions to the preshipment test for anaplasmosis. Although these three bulls were negative to that test, two of them gave positive reactions on repeated tests while in quarantine. The consignee refused to accept the shipment and returned it to the ranch of origin. Our experience with imports again emphasizes
the point made in our last report, that 'there must be a 60-90 day retest on all cattle entering a clean area, unless they originate in a test negative herd'."

Research on anaplasmosis is continuing at 12 State Universities and Experiment Stations and the Beltsville, Maryland, Laboratory of Agriculture Research Service.

Field trials are being conducted in at least six states. Several years work will be necessary to evaluate the results of these trials.

The production of anaplasmosis antigen at the Texas A & M experiment station has progressed during the past year. Sufficient antigen to conduct 1,292,070 tests has been produced. This amount has been approved or is in the process of approval as of this time. This approaches the goal of producing sufficient antigen to conduct 2,000,000 tests by July 1, 1960. The antigen is necessary for use in field trials, in the Hawaiian program, for diagnostic purposes and research. It is questionable that this amount will be adequate to meet the demands for these purposes.

The complement-fixation test has been accepted as recommended by the sub-committee appointed at the third National Research Conference on Anaplasmosis.

The characteristics of the etiological agent of the disease has been studied the past year with renewed interest revealing many interesting characteristics. However, the exact nature of the agent has not been determined.

The Committee recommends that emphasis be placed on the following:

1. Additional basis research, especially on etiology and pathology, is necessary in order that practical control and treatment for the disease may be developed.

2. Research and field trials be continued and expanded to include all the geographical areas of this country where the disease is a problem.

3. That a continuing and adequate supply of the test antigen be produced and made available.

4. That work be continued to develop improvements on the present test and explore the possibilities of more easily conducted tests.
THE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM


Washington, D. C.

Progress made during the past five years in the cooperative state-federal bovine brucellosis eradication program has established an enviable record of accomplishment. The rapidity with which the listing of Modified Certified Brucellosis Areas has grown during this period underlines the effectiveness of the program as now constituted. These advances also reflect a continued high industry support. It is obvious, of course, that no program of this nature can succeed without such support.

On a nation-wide basis, we are still faced with the problem of meeting all program service requirements. This situation has developed to the point where it is the primary limiting factor on the rate of progress that can be made. The momentum generated over the past few years should not be jeopardized through failure to continue the program at the maximum level possible.

With more and more states becoming completely qualified as Modified Certified Areas it becomes increasingly important that criteria for establishing and maintaining Certified Brucellosis-Free Areas be developed. Unless provisions for effectively designating such areas are provided there may be a tendency for the Modified Certified states to relax their efforts to achieve anything more than this status. There are still those who do not believe that brucellosis can be eradicated. Until conclusive evidence to the contrary is available, this element of doubt will persist. There is reason to believe that a significant number of counties throughout the country could qualify as bovine brucellosis-free areas at this time, even under the strictest possible interpretation of the designation.

With the estimated annual losses to the livestock industry caused by bovine brucellosis having been reduced from $100 million in 1947 to less than $30 million, we cannot afford to lose these advantages by lessening the effort that made them possible. Disregarding entirely the public health aspects of the disease, it is highly significant that all combined state and federal funds expended in this project since 1935 are the equivalent of only three and one-half years' losses at the 1947 estimated rate. On this basis alone, therefore, the value received for each dollar invested more than justifies continued effort being directed toward the eventual eradication of brucellosis from all susceptible livestock species.

* Chief Staff Officer, Brucellosis Eradication, Agricultural Research Service, United States Department of Agriculture.
DECLINE IN PROGRAM ACTIVITIES

During fiscal year 1959, significant reductions occurred in most field activities connected with the cooperative brucellosis eradication program. Some of the factors contributing to this decline are (1) the increasing number of Modified Certified Brucellosis Areas, (2) expanding utilization of the brucellosis ring test, and (3) decreased state-federal funds available for support of the program.

Table I shows a comparison of operations conducted in fiscal years 1958 and 1959.

TABLE I
TABULATED NATIONWIDE REPORT ON BRUCELLOSIS ERADICATION ACTIVITIES

<table>
<thead>
<tr>
<th>Activities</th>
<th>Fiscal Years</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1958</td>
<td>1959</td>
</tr>
<tr>
<td>Blood Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds—Lots</td>
<td>1,176,601</td>
<td>984,576</td>
</tr>
<tr>
<td>Reactor</td>
<td>108,560</td>
<td>81,226</td>
</tr>
<tr>
<td>Percent</td>
<td>9.2</td>
<td>8.2</td>
</tr>
<tr>
<td>Cattle</td>
<td>16,251,440</td>
<td>14,168,909</td>
</tr>
<tr>
<td>Reactor</td>
<td>260,322</td>
<td>214,331</td>
</tr>
<tr>
<td>Percent</td>
<td>1.60</td>
<td>1.51</td>
</tr>
<tr>
<td>Reactors Slaughtered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>97.7</td>
<td>94.9</td>
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<tr>
<td>Ring Tests</td>
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<tr>
<td>Herd Tests</td>
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<tr>
<td>Suspicious</td>
<td>164,224</td>
<td>103,987</td>
</tr>
<tr>
<td>Percent</td>
<td>9.38</td>
<td>6.13</td>
</tr>
<tr>
<td>Vaccinations</td>
<td>6,276,910</td>
<td>6,702,832</td>
</tr>
<tr>
<td>Certifications</td>
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<tr>
<td>New Counties</td>
<td>490</td>
<td>440</td>
</tr>
<tr>
<td>Counties Removed</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total Certified Counties</td>
<td>1,217</td>
<td>1,653</td>
</tr>
</tbody>
</table>

( ) Actual difference.

Blood Testing: For the period of July 1, 1958 through June 30, 1959, a total of 14.1 million blood serum agglutination tests for bovine brucellosis were conducted in slightly less than one million herds. This represents a reduction of 10.9 percent in the number of animal tests and 16.3 percent in the number of herds covered, as compared with the previous year. As might be expected, both cattle and herd infection rates were lowered still further during the year. As of June 30, 1959, the indicated level of animal and herd infections throughout the country were 1.5 percent and 8.2 percent respectively.
**Brucellosis Ring Testing:** The efficiency of the milk and cream ring test for detecting *Brucella* infected dairy herds has been confirmed each year since this procedure became a part of the official program. During the past year approximately 30 million cattle were represented in the 1.6 million herds screen-tested. With the 14.1 million blood tests conducted during the same period, there was the equivalent of nearly 35 million animal tests made during the year. Without the advantages of the ring test this level of coverage could not be approached with the limited manpower available. It is of interest to note also that ring test suspicious herds have been reduced from 27.0 percent in 1952 to 6.1 percent in 1959.

**Vaccination:** Available records indicated that we are now vaccinating about 52 percent of the eligible calves in the United States. While there has been a gradual increase each year in the number of calves vaccinated since Strain 19 vaccine was approved in 1941, there still is a need to expand this phase of the program. Until brucellosis is finally eradicated vaccination will continue to be an important factor in limiting the spread of infection. Every effort must be made to counteract the tendency for a relaxation of vaccination in the Modified Certified Areas. At this stage of the program, the benefits of vaccination are more valuable in these areas than ever before. It is hoped that the 6.7 million official vaccinations recorded for fiscal year 1959 will be increased in succeeding years until at least 75 percent of the eligible calves will be vaccinated each year.

**MODIFIED CERTIFICATION STATUS: COUNTIES**

Cooperative State-Federal Brucellosis Eradication Program

- Certified: 1,777 Counties (57%)
- No Area Work: 1,020 Counties (32%)
- Area Work in Progress: 355 Counties (11%)

Total Counties: U.S., P.R., V.I., Hawaii: 3,152

Data as of Nov. 30, 1959
As the program moves ahead toward the goal of eradication, the advantages of complete area work become more evident. Without the protection afforded by this type of operation, consistent progress would be difficult to maintain in many areas of the country.

At the end of fiscal year 1959, there were 1,656 Modified Certified Brucellosis Areas, including 19 complete states, Puerto Rico and the Virgin Islands. During the 12 months period ending June 30, 1959, a total of 439 counties were initially qualified. This compares with the 482 counties that were added in 1958.

As of November 30, 1959, the number of Modified Certified counties had increased to 1,777. At the same time, there were 355 other counties conducting area work leading directly to Modified Certification. On June 30, 1959, 492 counties were operating on a complete area-work basis. This reduction of 137 area-work counties within a five-month period reflects the shortage of state-federal funds available for program operations during the current fiscal year 1960.

PRELIMINARY RESULTS OF CULL AND DRY COW TESTING ENCOURAGING

The need for a screening device in range and semi-range areas, comparable to the brucellosis ring test in dairy states, has been recognized for a long time. It appears now that the so-called “cull and dry” cow testing program offers a solution to this problem. As adopted by the United States Livestock Sanitary Association and approved by the Agricultural Research Service, the “cull and dry” program provides for the effective recertification of range and semi-range areas with a minimum of inconvenience to owners.

During the past year considerable effort has gone into the implementation of such a program in the western states. In general, there is strong support for this procedure and provided the necessary cooperation is forthcoming no insurmountable obstacles to its successful application are anticipated. This opinion is supported by preliminary records covering these activities for the months of October and November 1959. For this limited period, 18 states indicated some degree of participation in the project. There were 29,240 back-tags applied and 39,914 blood samples collected from the type of animals involved. It was encouraging to note also that of the 120 reactors disclosed on these tests, 110 had been traced promptly to herds of origin. There is every reason to believe the “cull and dry” cow testing program can be developed into an effective procedure for recertifying range and semi-range areas. With proper application it should provide a better method of detecting infected herds than the alternate 20 percent test provision.

EFFECTIVENESS OF MODIFIED CERTIFICATION PROCEDURES FURTHER DEMONSTRATED

One of the primary incentives for Modified Certification area work, especially in range sections of the country, is preferential treatment of cattle
moving from areas that have achieved this distinction. Unfortunately, many of the importing states have been reluctant to grant this recognition and as a consequence initial certification of some range areas has been delayed. Unless this situation is corrected, it may become increasingly difficult to complete and maintain the Modified Certification program.

At the present time, 21 states grant special privileges to cattle originating in fully qualified Modified Certified Brucellosis Areas. An additional 12 states have indicated a sympathetic understanding of the problem and are willing to consider necessary changes in their existing laws and regulations.

As a means of determining the brucellosis status of these animals, tests have been made during the past year on a total of 65,466 interstate movements from Modified Certified and non-certified areas. In all instances these tests were conducted within 60 days following arrival of the cattle at destination. Of the 44,008 animals tested from Modified Certified Areas, there were only 26 reactors found, or one in each 1,693 tested. In contrast, out of the 21,457 animals tested from non-certified areas, 176 reactors were disclosed, or one in each 122 tested. There is no question but that the advantages to be attained for the nation-wide program by providing freer movements of cattle from Modified Certified Areas far outweigh any negligible risk involved.

**CONTROL VERSUS ERADICATION**

Regardless of the excellent progress made during the past few years, there are still those who refuse to believe that brucellosis can be eradicated. It is difficult to reconcile this point of view with the clear indications that eradication is being accomplished at an ever-increasing rate. Of the many facts that could be cited in support of the eradication concept, a few are discussed below:

- There are several hundred thousand previously infected herds throughout the country that have been freed of brucellosis.
- Animal infection rates have been reduced from 11.5 percent in 1934 to 1.5 percent in 1959.
- As of November 30, 1959, there were 1,777 counties in which the incidence of infection had been reduced to not more than one percent of the cattle in five percent of the herds.
- There are 154 counties in 27 states which have shown no evidence of bovine brucellosis on the last recertification tests. In some of these, no reactors have been disclosed for more than six years. There are numerous other counties throughout the nation which were practically free of the disease when last tested and in which all known infected animals now have been identified and removed from the herds involved.
- The incidence of human brucellosis has been reduced from 6,321 reported cases in 1947 to only 721 in 1959.

Faced with these and many other similar facts, the ultimate goal of eradication is one that can be accepted as being fully realistic. Results over the past five years demonstrate conclusively that available tools and pro-
State-federal procedures can accomplish the eradication of brucellosis from our livestock population if we so desire. Reversion to a control program only at this advanced stage of progress is inconceivable.

FUTURE PROSPECTS

State-federal funds available for support of the cooperative brucellosis eradication program are 17.3 percent less in fiscal year 1960 than were provided during 1959. With the intimate relationship that exists between available funds and progress, the program will be retarded this year. At the present time, service requirements are continuing at about the same level as existed throughout fiscal year 1959.

Barring unforeseen difficulties, it is anticipated that approximately 300 new counties will qualify as Modified Certified Areas this year. During fiscal year 1959, 439 initial county certifications were listed. The greatest change expected during 1960 will be in the number of area-work counties. As of December 1, 1959, there were only 350 counties engaged in activities of this nature. On July 1, 1959 nearly 500 counties were operating on the same basis. With limited funds available for the program this year it has been necessary to limit the number of new counties accepted for area work. It is estimated that operations in nearly 200 qualified area work counties has been delayed indefinitely because of fund limitations.

With the current reduced level of state-federal support, completion of the Modified Certified Area program throughout the country will require about nine years longer than had been anticipated at the 1959 rate of progress. A recent study shows that 16 states have been forced to extend the time originally considered necessary to achieve the complete Modified Certified status.

GENERAL COMMENTS

More progress has been made over the past five years than during any similar period since the cooperative state-federal brucellosis eradication program was inaugurated.

Existing financial problems are retarding progress at a time when more eradication is being purchased for each dollar spent than ever before.

It is essential that full support be given the cull and dry cow testing program wherever it is applicable. This procedure can provide a practical and efficient solution to the problem of recertifying range areas and will contribute to the eventual eradication of brucellosis from all sections of the country.

With 57 percent of all counties in the United States, Puerto Rico and the Virgin Islands qualified as Modified Certified Brucellosis Areas, it is urgent that provisions be developed for the establishment and maintenance of Certified Brucellosis-Free Areas. From the standpoint of infection, it appears that approximately 500 counties are in a position to move rapidly toward this status.
The investment already made in the brucellosis eradication program is paying worthwhile dividends. Within a period of 10 years there has been a 75 percent reduction in the estimated annual economic losses caused by this disease.

While the outlook for the brucellosis eradication program is reasonably good, its successful completion will require the dedication of everyone concerned to the principles of eradication as opposed to the doctrine of control only. We are on the threshold of achieving the most remarkable success ever recorded in the history of livestock disease eradication programs. Under the circumstances, therefore, it is difficult to believe this opportunity will be lost through lack of interest.
THE RESULTS OF ELEVEN YEARS' VACCINATING WITH STRAIN 19


Official state-wide calfhood vaccination with strain 19 brucella organisms started in California, January 2, 1948. This was in accordance with a law passed by the 1947 legislature, which made vaccination compulsory in all female dairy calves, and provided for voluntary vaccination of all beef calves and male dairy calves.

California has now gone through nearly 12 years of vaccination. We had from 9 to 10 years' vaccination behind us when the new area eradication program started October 1957—a little over two years ago.

The services of from 300 to 375 accredited practicing veterinarians have been used on a contractual basis, paying 75 cents a head for vaccinating, tattooing and reporting, and the state furnished the vaccine. The program was organized and supervised out of 10 district offices by approximately 75 federal and state veterinarians in conjunction with their regular disease control activities, such as tuberculin testing.

CONTROL OF VACCINE—All vaccine used in the program is purchased by the state under specifications requiring 8½ billion viable organisms per cubic centimeter. Each serial lot is tested by the state prior to release. Only single dose containers have been used, and a change was made from liquid to lyophilized vaccine soon after the improved product was on the market.

GETTING THE JOB DONE—Several years after the program started, it became necessary to use the services of the federal and state veterinarians to vaccinate small and isolated lots not readily accessible to the practicing veterinarians. To attain better coverage of small lots that should have been vaccinated by the practicing veterinarians, it also became necessary to offer an additional monetary inducement. Therefore, arrangements were made for the federal government to pay for vaccinating small lots of five calves or less by reimbursing the practicing veterinarians $2.50 for each stop made, in addition to the regular fee of 75 cents a calf. This stimulated vaccination noticeably in these small lots and increased the coverage considerably.

The program has cost the state about $400,000 annually, exclusive of administrative expenses.

Estimates were made at various times that about 65 percent of the eligible beef calves and 80 to 90 percent of the eligible dairy calves were being vaccinated, although it took several years to attain this degree of coverage. It finally took a regulation in 1954, requiring either vaccination or a negative

* Division of Animal Industry, California Department of Agriculture, Sacramento, California.
test before dairy cattle could be moved, to obtain better coverage of dairy calf vaccination. As an additional stimulus to vaccination, the new cooperative area eradication program requires that all eligible calves, both beef and dairy, be regularly vaccinated in order for the area to maintain the modified certified brucellosis status. There was a 10 percent increase in vaccination the first year after the eradication program started in the fall of 1957. There was a 34 percent increase in beef cattle owners vaccinating in 1958 over the previous year. This is interesting for the reason that 1958 was the first full year of the eradication program—indicating that the certification program has had a stimulating effect on vaccination and that the coverage had not been good in the beef calves on a voluntary basis.

These historical events are pointed out to show that you do not get all of the calves in a state properly vaccinated by just waving a wand—voluntary programs will not get the job done—it takes money, many years of hard work, proper supervision and adequate, well-enforced laws and regulations requiring vaccination of all calves.

Graph I shows the progress made each year in the number of calves vaccinated and reflects the various historical events just cited.

GRAPH I

BEEF AND DAIRY CALF VACCINATIONS 1948 - 1958

- Total calves
- Dairy calves
- Beef calves
ELEVEN YEARS' VACCINATING WITH STRAIN 19

THE RESULTS OF VACCINATION

Those who developed strain 19 vaccine adequately demonstrated, under controlled experiments, that its efficiency in warding off infection depended, fundamentally, directly on the degree of exposure to virulent brucella organisms. From a regulatory standpoint we know there are other factors that influence the results under field conditions. Therefore, an effort is made in this discussion to demonstrate (1) what vaccination has accomplished statewide, (2) the effectiveness in cattle of the beef breeds under semi-range conditions where the vaccination was done voluntarily, and (3) the results on dairy cattle in the highly intensified dairy sections of the state where vaccination of dairy calves was mandatory, in (a) closed herds and (b) open herds. Closed herds are those raising all of their replacements and open herds are those that buy most of their replacements.

THE RESULTS STATE-WIDE

Prior to 1948 when the calf-vaccination program started, it was estimated that the incidence of brucellosis infection in the state was about 17 percent to 18 percent in dairy cattle and 7 percent to 9 percent in beef cattle. Prior to 1957, two years ago, there had been no official state-wide eradication program.

After 9 to 11 years of vaccinating, and in the last two years since the eradication program started in October 1957, 33,836 herd blood tests, representing 2,549,647 cattle, have been made. 10,320 reactors have been found. The testing was done in cooperation with the Animal Disease Eradication Division of the United States Department of Agriculture.

From a state-wide standpoint our records indicate that intensive, well-supervised vaccination over an 11-year period, compulsory in dairy calves but voluntary in beef calves, has been instrumental in reducing the incidence of the disease to a very low point. It has laid a good solid foundation for an eradication program, but it has not eradicated the disease. We have certified 24 counties and 16 more should be certified by June 30, 1960.

We were not in the testing program very long until it was realized that, generally speaking, vaccination had done a pretty good job of reducing the infection to a very low point, but infection was being found in scattered herds here and there.

THE RESULTS IN BEEF CATTLE UNDER SEMI-RANGE CONDITIONS

At the time of the initial area brucellosis tests in herds of the beef breeds in Lassen, Modoc and Siskiyou Counties of the extreme northeastern section of California, data became available from herd test records suitable for evaluating the effect of vaccination on the infection rate for both herds and cattle. The livestock industry in these counties is largely concerned with the production of beef under semi-range conditions. Most of the herds in these counties are on the range for approximately six months of the year and are collected in the fall and fed hay under fence during the winter months, thereby falling into the classification of semi-range herds.
Brucellosis test records from these three counties were selected for this study because calfhood vaccination had been extensively practiced for a period of approximately 10 years. The vaccination records available for the three counties indicate approximately 25,000 calves were vaccinated each year. The total herd population over four months of age is approximately 150,000 head. It should be pointed out that many of the herds tested did not start a vaccination program until a year or two before the area testing program went into effect. In these cases cattle of an age eligible for test were non-vaccinated, even though the young stock had been vaccinated for the past few years.

Initial herd tests in the three counties encompassed 1,380 herds with 67,762 individual blood tests. There were 928 reactors found in 186 infected herds. The herd infection rate was 13.4 percent. The reactor rate in cattle tested was 1.4 percent and 0.7 percent when calculated on the basis of the total herd population.

In this study five separate herd categories were set up, based on the degree of vaccination, as follows: (1) Herds with no vaccination history, (2) herds with 1 to 25 percent of the cattle vaccinated, (3) herds with 26 to 50 percent of the cattle vaccinated, (4) herds with 51 to 75 percent of the cattle vaccinated, and (5) herds with 76 to 100 percent of the cattle vaccinated. Table I provides the basic data for each of the categories studied.

### TABLE I

<table>
<thead>
<tr>
<th>Percent Vaccinates</th>
<th>Herds Tested</th>
<th>Cattle Tested</th>
<th>Cattle Infected</th>
<th>Cattle Tested Reactors</th>
<th>Cattle Tested Non-vac.</th>
<th>Cattle Tested Suspects Vnc.</th>
<th>Cattle Tested Vac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>176</td>
<td>12,150</td>
<td>20,966</td>
<td>34</td>
<td>0</td>
<td>256</td>
<td>0 362</td>
</tr>
<tr>
<td>1- 25%</td>
<td>94</td>
<td>7,296</td>
<td>14,833</td>
<td>27</td>
<td>5</td>
<td>212</td>
<td>108 492</td>
</tr>
<tr>
<td>26- 50%</td>
<td>123</td>
<td>9,929</td>
<td>17,865</td>
<td>29</td>
<td>8</td>
<td>120</td>
<td>155 497</td>
</tr>
<tr>
<td>51- 75%</td>
<td>119</td>
<td>12,984</td>
<td>26,356</td>
<td>28</td>
<td>3</td>
<td>74</td>
<td>179 332</td>
</tr>
<tr>
<td>76-100%</td>
<td>137</td>
<td>14,787</td>
<td>35,691</td>
<td>17</td>
<td>7</td>
<td>28</td>
<td>194 104</td>
</tr>
<tr>
<td>Total</td>
<td>649</td>
<td>57,146</td>
<td>115,711</td>
<td>135</td>
<td>23</td>
<td>690</td>
<td>636 1,787</td>
</tr>
</tbody>
</table>

Data assembled from initial tests only.

### TABLE II

| Herds: Brucellosis Infection Status in Relation to Herd Vaccination Practices* |
|---------------------------------|-----------------|----------------|-----------------|-----------------|
| Herd Vaccinated | Herds Tested | Cattle Tested | Cattle Tested | Infected Herds Number |
| None              | 176          | 12,150        | 34             | 19              |
| 1- 25%            | 94           | 7,296         | 27             | 29              |
| 26- 50%           | 123          | 9,929         | 29             | 24              |
| 51- 75%           | 119          | 12,984        | 28             | 24              |
| 76-100%           | 137          | 14,787        | 17             | 12              |

*Initial beef herd test data from Lassen, Modoc, and Siskiyou Counties. Herds of less than 10 eligible cows excluded.
Table II was prepared to show the herd infection rate in relation to vaccination practice. Examination of this data indicates that the herd vaccination status did not markedly reduce the herd infection rate. This observation does not portray the true herd infection picture since examination of the test records usually revealed large numbers of reactors in non-vaccinated infected herds, whereas very small numbers of reactors were found in relatively well-vaccinated herds.

**TABLE III**

Beef Cattle: Brucellosis Reactor Rate in Relation to Herd Vaccination Practice

<table>
<thead>
<tr>
<th>Herds: Vaccinates</th>
<th>Cattle Tested</th>
<th>Percent Reactors*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12,150</td>
<td>0</td>
<td>2.1</td>
</tr>
<tr>
<td>1-25%</td>
<td>7,296</td>
<td>0.5</td>
<td>3.3</td>
</tr>
<tr>
<td>26-50%</td>
<td>9,929</td>
<td>0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>51-75%</td>
<td>12,984</td>
<td>0.04</td>
<td>1.5</td>
</tr>
<tr>
<td>76-100%</td>
<td>14,787</td>
<td>0.06</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Rates calculated as follows:
1. Vaccinated reactor rates based on percentage of vaccinates tested.
2. Non-vaccinated reactor rates based on non-vaccinates.

**GRAPH 11**

**BRUCELLOSIS REACTOR RATE**
Examination of Table III and Graph II would indicate that in a well-vaccinated herd one would expect a 10-fold reduction in the percentage of reactors when compared to non-vaccinated herds. In fact, the efficiency of the vaccine, as shown by this data, implies that complete vaccination of beef cattle within a geographic area might suppress the disease nearly to the point of eradication. Experience in this area has, however, shown that complete vaccination coverage is difficult to attain.

### TABLE IV

**Beef Cattle: Brucellosis Suspect Rate in Relation to Herd Vaccination Practice**

<table>
<thead>
<tr>
<th>Herds: Percentage Vaccinates</th>
<th>Cattle Tested</th>
<th>Percent Suspects* Vaccinated</th>
<th>Non-Vaccinated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12,150</td>
<td>0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1- 25%</td>
<td>7,296</td>
<td>11.4</td>
<td>7.7</td>
<td>8.2</td>
</tr>
<tr>
<td>26- 50%</td>
<td>9,929</td>
<td>4.1</td>
<td>8.1</td>
<td>6.6</td>
</tr>
<tr>
<td>51- 75%</td>
<td>12,984</td>
<td>2.2</td>
<td>7.0</td>
<td>4.0</td>
</tr>
<tr>
<td>76-100%</td>
<td>14,787</td>
<td>1.5</td>
<td>5.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Rates calculated as follows:
1. Vaccinated suspect rates based on percentage of vaccinates tested.
2. Non-vaccinate suspect rates based on non-vaccinates.

Table IV shows the suspect rate in relation to the herd vaccination practice. Evaluation of this data does not indicate vaccination titers are a primary cause for the presence of suspect animals. In fact, the suspect rate in well-vaccinated herds appeared to recede. However, this observation may be a reflection of a reduction in herd infection status rather than in the suspect picture alone.

### THE RESULTS IN DAIRY CATTLE UNDER CLOSED AND OPEN HERD CONDITIONS

**CLOSED HERD AREAS**—Following are observations made in the central valley area, known as the Modesto district, where dairying is highly intensified. The herds averaged about 55 cattle, the winter climate is mild and pasturing is practiced to some extent the year around.

The majority of herds in this area are closed herds. Replacements are raised and are very nearly 100 percent vaccinated.

Initial tests on 3,700 herds, representing 221,000 cattle, revealed:

- 74.3 percent of the herds were BRT negative.
- 50.0 percent of BRT suspect herds were blood test negative.
- 11.2 percent of the herds had blood test reactors.
- 1.41 percent of the total cattle were reactors.

From these test results it is evident that, in the great majority of the herds, vaccination has prevented or eliminated the disease. As an example of this we may cite the combined record of three large well-vaccinated herds containing 4,300 cattle and having a history of infection prior to the vaccination
program. All were negative to the initial BRT test and have passed a second negative test.

Further evaluation of vaccination by examination of individual herd records shows us that there are three categories of herds in which vaccination alone is not sufficient.

The first group containing most of the infected herds, are herds in which the disease is present but in which the symptoms are practically controlled. These herds clean up rapidly. For example, a 236 cow herd, well-vaccinated, had 34 reactors. Difficult breeding in many of the reactors was the only symptom. A second blood test was negative.

The second group are well-vaccinated herds to which susceptible additions are made. One herd of 419 cows in this group recently added 200 non-vaccinated negative animals from another state. Forty-four reactors have been taken from this herd in the past year. Abortions occur in both the original as well as the added cattle. The herd is showing little tendency to clean up on the blood test.

Finally, there is a very small group of herds in which field breaks of unknown origin occur despite good vaccination. For example, a herd of 193 cows with no history of infection had one reactor in December 1958. Apparently the disease was just starting, as 18 reactors have been found in the herd in the past nine months, most of which were aborters.

Herds in these last two groups emphasize the fact that further methods of controlling the disease than vaccination still need to be considered.

Seventy-five percent of the closed herds became negative after one blood test, 25 percent required two to three tests; it required an average of one and one-half tests to attain a clean status. Seventy-five percent of the open herds required three or more tests, and an average of three tests was required to attain a clean status for the group.

Open Herd Areas—The following data was taken from the results of initial area tests in Santa Clara County, in the central coast area:

One hundred forty commercial grade A milk producers, averaging 160 cows per herd, operate on an average of three acres of ground. They resemble a feed lot type of operation, and replacements, for the most part, are purchased. The exposure to brucellosis has been heavy and varied, and the immunity given by strain 19 vaccine is constantly challenged.

Blood tests were made on 19,932 cattle. Approximately 50 percent of the herds in the county were ring test negative while 74.3 percent of the closed herds were BRT negative. Of the 50 percent BRT suspicious open herds, 98 percent had blood test reactors while only 11.2 percent of the closed suspect herds had blood test reactors. The reactor rate in the county was low, being 2.25 percent, but almost twice of that in the so-called closed herds.

Eighty-eight percent of the cattle under test were vaccinates. Only 1.35 percent of the vaccinated cattle reacted (236), while 7.04 percent of the non-vaccinated cattle reacted (172). Only 7.35 percent of the vaccinates were suspicious, while 17.08 percent of the non-vaccinates were suspicious. While vaccine did not show as good protection in the open as in the closed herds,
it did show high value in the open herds, closely confined, where exposure challenge to brucellosis has proven to be most severe.

Subsequent tests show that it required an average of about two and one-half blood tests to clean the herds in this open herd district, as compared to one and one-half tests in the closed herds.

**SUMMARY**

1. A study was made of the results of a brucellosis testing program following 11 years of vaccination with strain 19 vaccine, compulsory in dairy calves and voluntary in beef calves.

2. Attaining satisfactory coverage of resident animals as well as of herd additions proved to be one of the greatest problems in a vaccination program. The value of vaccination is unquestioned when it is considered that conservative estimates, prior to the vaccination program, placed the infection rate at 17 percent to 18 percent in dairy cattle and 7 percent to 9 percent in beef cattle. This report would indicate that the infection rate in California dairy cattle was reduced to an apparent 2 percent and in semi-range cattle to an apparent 0.7 percent by vaccination alone. The 24 counties certified to date were essentially certified on an initial area test with a limited amount of retesting required in infected herds. The infection rate was reduced to 0.4 percent in a two-year period. We do not believe this reduction of infection could have been attained in California, as it has been, without the aid of disease-suppressive factors provided by the vaccination program.

3. In beef cattle in the semi-range areas, the degree of infection was directly proportional to the degree of vaccination. The herd infection rate was 50 percent lower in herds where 75 percent to 100 percent of the animals were vaccinated as opposed to the non-vaccinated or low level vaccinated classes. The effectiveness of vaccination in the beef cattle leads to the conclusion that a well-enforced vaccination program could be expected to maintain an infection rate low enough to qualify semi-range areas as modified certified brucellosis areas, but would probably not eradicate the disease. A voluntary program over a period of 10 years had reduced the infection rate to a very low point but not sufficient to "certify" the area without resorting to official testing and removal of reactors.

4. In closed dairy herds, where vaccination was compulsory, strain 19 vaccine was remarkably effective in eliminating the disease in many herds but some testing and removal of reactors was found necessary in approximately 11 percent of the herds. In the open herds where outside replacements were introduced a much higher degree of infection was found. These herds required two and one-half to three blood tests to attain a clean status as opposed to one and one-half such tests in the closed herds. After some 11 years of compulsory calfhood vaccination of dairy type female cattle in California, bovine brucellosis, with or without abortions, still prevails in dairy herds where new individuals are routinely added to the herds and where cattle are maintained closely confined on limited acreage.
SUMMARY OF CONTROLLED RESEARCH WITH STRAIN 19

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

Several meetings have been held during the past year to discuss the future of the present brucellosis program in this country. The opinions expressed on how to conduct this program have been many and varied. There are those who want a program of vaccination only, and those who believe it is necessary to use all of the procedures at our command. What we wish to do depends on whether we are interested in eradication or control of bovine brucellosis. If it is eradication, we need to apply all proven procedures with our best judgment; however, if we are interested in control only, it can be accomplished in varying degrees depending on the procedure employed. The one procedure most frequently suggested is vaccination with Strain 19 vaccine.

Research on Strain 19 and its application has been more comprehensive than that on most biologics used for immunization. Most of the information developed from controlled research, field trials, and program application since 1941 has been verbally presented or published at different times during the past 30 years. Nevertheless, complacency resulting from the present low economic impact of bovine brucellosis, and variable opinions concerning the value and limitation of Strain 19 vaccine make it desirable to review, summarize, and critically evaluate the pertinent data so that Strain 19 vaccination can be placed in its proper prospective. This information must be considered objectively if we hope to carry on a program that will progress toward eventual eradication of brucellosis from our cattle population.

Strain 19, which is a member of the species Brucella abortus, was one of the first modified microorganisms to be used extensively as an immunizing biologic in this country. The stability of its modified status is maintained by selection of the desired colonial form, propagation under selective environmental conditions, and preservation of desirable characteristics by either desiccation or freezing. The outstanding characteristics of the smooth-intermediate (SI) colonies of Strain 19 are: (1) stable low pathogenicity, (2) relatively high immunogenicity, and (3) moderate antigenicity. Although its characteristics of low pathogenicity was reported as early as 1930 by Buck (2), it was not until 1941 that it was critically tested by Mingle et al. (9), and 1949 by Taylor and McDiarmid (12).

The consistency of vaccines produced with Strain 19 is dependent on a number of carefully controlled procedures. Only seed cultures with a very low dissociation index are selected to supply producers of vaccine in the United States and in foreign countries. New cultures are supplied every three or four months. Specific methods of production are recommended.

* Animal Disease and Parasite Research Division, National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Md.
Strain 19 vaccine is marketed in either liquid or desiccated form and in single or multiple dose bottles. All vaccines should be held at refrigeration temperature (4 to 7°C) until used so that maximum viability will be maintained. Desiccated vaccine should be used immediately after reconstitution to liquid form. Although the viability of desiccated vaccine is affected less than liquid vaccine by adverse environmental conditions encountered in shipping and storage, it can be reduced below an effective immunizing level or completely destroyed by prolonged exposure to temperatures higher than 4 to 7°C.

Representative samples of each commercially produced serial lot of vaccine are tested by Laboratory Services, and these lots must meet certain specifications before they are approved for use in the field. Determinations are made for viability, colonial characteristics, contamination, and hydrogen-ion concentration. All serial lots of vaccines must contain a minimum of 10 billion viable Brucella per ml. and at least 85 percent SI colonies and must be free from contaminants.

The objective of vaccination is to reduce the susceptibility of the host by increasing resistance to disease. The process of vaccination, however, does not insure immunity. Moreover, the degree of immunity produced by vaccination with viable Strain 19 depends upon the response of each animal. This response may vary from no measurable immunity to complete protection; consequently, the expression is often used that the immunity produced by Strain 19 vaccine is relative.

Considerable research has been done to determine the effect of dose, method of administration, and age of animal at the time of vaccination upon the immunity induced with Strain 19 vaccine. The original concentration of viable Strain 19 organisms was established at 10 billion per ml. because maximum viability could be maintained best at this level in liquid vaccine. Although this concentration has been maintained throughout the years, it has little or no significance in desiccated vaccine. The optimal dose of viable Strain 19 organisms has not been established, and it is very questionable if it is desirable to do so because of the variable immunologic response exhibited by the host. Field application as well as research, however, has demonstrated that a dose of 50 billion viable Strain 19 organisms will produce a serviceable immunity to brucellosis.

Unless the volume of the vaccinal dose is changed, the subcutaneous method of administration is more practical than either the intracutaneous or intracaudal methods. When the dose of vaccine is the same, the method of administration has no significant effect upon the degree of immunity produced or the degree and persistence of vaccinal titers. Prolonged swelling and subsequent induration after subcutaneous administration of Strain 19 vaccine can be practically eliminated if the injection is made at the upper one-third of the shoulder or immediately posterior to the shoulder.

Although the age of the animal at the time of vaccination has more influence upon the sero-agglutinin response than either the dose or the method of vaccination, it has no effect on the immunologic response. It has been clearly demonstrated that cattle vaccinated after sexual maturity do not develop any
greater degree of protection than those vaccinated during calfhood. Consequently, there is no legitimate reason for vaccinating animals after they have become nine months of age, except for an occasional unusual circumstance.

Since Strain 19 vaccine will be frequently mishandled, which causes a reduction in viability, and a smaller number of viable organisms than 50 billion is capable of producing immunity, the recommended 5-ml. dose and the subcutaneous method of administration provide the best probability of obtaining serviceable protection in cattle against brucellosis.

Other factors that have a significant influence on the effectiveness of Strain 19 vaccine in preventing bovine brucellosis are the virulence of the infecting agent and the amount of exposure received by the animal. Table I shows the relationship of infection to various amounts of exposure with the highly virulent Br. abortus Strain 2308. This is a clear demonstration of a decreasing effectiveness of vaccination associated with an increasing amount of exposure. The table further shows that there was a difference between infection and abortion rates which is a clear demonstration of a difference in degree of immunity among some vaccinated animals. Regardless of the relatively high infection rates in vaccinated animals that received the larger exposure doses, the rates were much less than those in the unvaccinated animals. The infection rates associated with the 700 thousand exposure dose more closely parallels those associated with natural exposure that occurs in a herd with an incidence of approximately 50 percent infection; consequently, 700 thousand virulent Br. abortus has been the exposure dose most frequently used in controlled experiments with Strain 19 vaccination since 1947. The practical application of this data is that exposure to infection must be minimized to realize the greatest potential from vaccination. This can be done by removal of reactors and employment of good sanitary and management practices.

<table>
<thead>
<tr>
<th>Exposure Dose</th>
<th>254 Vaccinates</th>
<th>210 Non-vaccinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection Percent</td>
<td>Abortions Percent</td>
</tr>
<tr>
<td>350,000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>700,000</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>15,000,000</td>
<td>61</td>
<td>47</td>
</tr>
<tr>
<td>25,000,000</td>
<td>57</td>
<td>44</td>
</tr>
<tr>
<td>75,000,000</td>
<td>61</td>
<td>45</td>
</tr>
<tr>
<td>100,000,000</td>
<td>71</td>
<td>52</td>
</tr>
<tr>
<td>Natural</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Natural=Contact exposure to infected cattle within a herd where the incidence of brucellosis was maintained at 50 percent or greater.

A compilation of all data from research at the National Animal Disease Laboratory indicates that 65 to 75 percent of the vaccinated animals will be
C. A. MANTHEI

completely protected against most kinds of exposure to virulent *Br. abortus*. Although the remaining 25 to 35 percent of the vaccinated animals become infected, many do not show clinical signs, such as abortion. This evidence shows there was partial protection in a relatively large percentage of animals other than those that were completely protected. This is comparable to the field results obtained in the states of Montana (10) and California (11).

The length of time that immunity would remain serviceable in cattle vaccinated as calves was somewhat controversial until the past 10 years. Table II shows the trend of immunity induced in different age groups of cattle that were vaccinated as calves. The detailed results of Experiment 1 were reported in 1951 (7) and those of Experiment 2 in 1956 (5). Regardless of past opinions to the contrary, all of the evidence shows that there is not a decrease in immunity associated with an increase in age of the vaccinated animal. These findings are very similar to those reported by McDiarmid in 1957 (8) and by Gregory in 1958 (6). This is not surprising since we are dealing with an immunity that follows recovery from infection induced with the low pathogenic Strain 19. Furthermore, age does not appear to be the factor associated with prolonged vaccinal immunity, because Goode et al. (4) were unable to show any difference in susceptibility of cattle of different ages to virulent *Br. abortus*.

### TABLE II

*Duration of Immunity to Brucellosis in Calf-Vaccinated Cattle*

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Percent Infection</th>
<th>Percent Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>52.4</td>
<td>52.4</td>
</tr>
<tr>
<td>3 years</td>
<td>44.4</td>
<td>27.8</td>
</tr>
<tr>
<td>4 years</td>
<td>52.9</td>
<td>29.4</td>
</tr>
<tr>
<td>5 years</td>
<td>15.8</td>
<td>21.1</td>
</tr>
<tr>
<td>6 years</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Average</td>
<td>37.6</td>
<td>30.6</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>38.3</td>
<td>25.0</td>
</tr>
<tr>
<td>3 and 4 years</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>5 and 6 years</td>
<td>26.3</td>
<td>26.3</td>
</tr>
<tr>
<td>7 and 8 years</td>
<td>11.3</td>
<td>11.8</td>
</tr>
<tr>
<td>9, 10 and 11 years</td>
<td>13.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Average</td>
<td>20.5</td>
<td>15.1</td>
</tr>
</tbody>
</table>

* All animals were vaccinated as calves and exposed at age indicated to 25,000,000 or more virulent *Brucella abortus*.

† All animals were vaccinated as calves and exposed at age indicated to 700,000 virulent *Brucella abortus*.

This brings us to the question of need and practicability of revaccination. If we accept the results of experiments on duration of immunity, there is definitely no suggestion of need for revaccination. Moreover, there certainly is very little evidence to suggest that the immunity of cattle previously vac-
cinated with Strain 19 can be reinforced to a significant degree. Neither Berman et al. (1) nor Gilman and Hughes (3) disclosed evidence of any benefit from revaccination. Although the work of McDiarmid (8) showed that a slight benefit resulted from revaccination, he did not consider it sufficient to make such a procedure practical. Gregory (6) also concurs in this belief.

What effect does Strain 19 vaccination have on the course of brucellosis in cattle? The majority of cattle owners, regulatory officials, and research workers associated with the brucellosis problem are aware that vaccination has practically eliminated the clinical disease and materially reduced the number of animals with udder infection. This suppression of clinical signs in cattle, which are not completely protected against any specific degree of exposure, should not lead to complacency because it does not mean that the disease has been eradicated. Some animals will harbor an insidious infection that could be the cause of recurrence of clinical brucellosis under certain conditions. It is not particularly uncommon for vaccinated animals to give birth to full-term living calves and yet have uterine infection. A more common condition found in partially immunized animals is the presence of infection in the mammary gland or the localization of infection in some remote organ or lymph gland within the body. Partial immunity also has a tendency to suppress bacteremia and to prolong the development of the diagnostic sero-agglutinin titer. There is no question that animals with genital infection are the most serious source of exposure to other cattle, but those with udder infection likewise can be a source of exposure. In calves that are permitted to nurse such animals or are pail fed contaminated milk, many viable Brucella organisms pass through the digestive tract and contaminate both feed and water. Consequently, suppression of clinical brucellosis is not only a hidden menace to profitable cattle production but to public health.

Strain 19 is incapable of curing cattle infected with brucellosis. Furthermore, it will not alter the natural course of the disease in such animals.

Before closing the discussion on vaccination, it should be emphasized that the greatest benefits from Strain 19 vaccination can be realized only if every effort is made to vaccinate as many calves as possible. This will materially decrease the spread of brucellosis, which will be reflected in a decrease in cost and in time to eradicate the disease. Field data have shown that vaccination will reduce animal infection approximately 80 percent and herd infection approximately 20 percent in areas where a majority of calves are vaccinated.

One would be remiss in his obligation if a short discussion of diagnostic procedures were not included in this presentation.

There have been and will be differences of opinion about the sero-agglutination test (blood test) for the diagnosis of brucellosis in the individual animals. Most of these differences have been associated with the costs involved and the accuracy of the test. The best estimate of accuracy by persons who have made a thorough study of this test is that it will identify 95 percent of the infected animals with a single test and at least 99.5 percent with a 30- to 60-day retest of infected herds.
Results from the field show that the Brucella milk ring test (BRT) conducted every six months is equally as efficient as are annual area blood tests in locating infected dairy herds. Moreover, it has the advantages of locating foci of infection more frequently and at less cost. There does not appear to be any apparent reason why the cull and dry method cannot be just as great a forward step to the beef cattle industry as the BRT has been for the dairy cattle industry.

Although we recognize the value of calf vaccination, the sero-agglutination test, and the BRT, we also have to consider other procedures to handle the small number of problem herds. Some work has already shown that serological milk tests and cultural procedures can be used to a distinct advantage. The serological milk tests most frequently used are the whey agglutination or the skim milk agglutination tests that employ the BRT antigen.

SUMMARY

1. Strain 19 vaccine is a fragile biologic, which requires specific handling to insure maximum viability.
2. A large measure of success attained with calf vaccination is dependent on the administration of the recommended dose of viable Strain 19 organisms.
3. The immunity produced with a fully potent vaccine is relative, principally because of differences in the immunologic response of individuals.
4. The effectiveness of Strain 19 vaccination is increased as the degree of exposure to virulent Brucella abortus is decreased.
5. Calves should be vaccinated at ages from four through eight months to prevent vaccinal titers from interfering with interpretation of the sero-agglutination test.
6. The degree and duration of immunity produced with calfhood vaccination is equal to that produced with adult vaccination.
7. Vaccinal immunity of cattle does not decrease as the age of the animals increases.
8. There is not any apparent need for revaccination, and it is neither desirable nor practical.
9. Vaccination with Strain 19 suppresses clinical brucellosis, but it does not always prevent infection.
10. Approximately 65 to 75 percent of the calf vaccinated cattle will have complete protection against most kinds of exposure to brucellosis, and the remaining 25 to 35 percent will have various degrees of protection.
11. Vaccination will reduce animal infection approximately 80 percent and herd infection approximately 20 percent in areas where a majority of heifer calves are vaccinated.
12. Strain 19 vaccination alone will control but will not eradicate bovine brucellosis.
13. Strain 19 is incapable of curing bovine brucellosis, and it does not alter the course of the disease when injected into infected animals.
14. The sero-agglutination test, the Brucella milk or cream ring tests, and the whey or skim milk agglutination test are valuable and necessary procedures for the control and eventual eradication of bovine brucellosis.
SUMMARY OF CONTROLLED RESEARCH WITH STRAIN 19

REFERENCES


Montana cattlemen wanted to know the relative protection afforded by Strain 19 Brucella vaccine under conditions of beef cattle husbandry as practiced in the State of Montana. Data was assembled from the initial area brucellosis test charts of herds of the beef breeds. A total of 951,014 cattle were tested in 21,418 herds, of which 255,757 animals (26.89 percent) had been vaccinated against brucellosis, while 695,257 (73.11 percent) had not.

Separate herd categories were set up as follows: (1) No vaccination, (2) one to 25 percent of cattle vaccinated, (3) 26 to 50 percent of cattle vaccinated, (4) 51 to 75 percent of cattle vaccinated, (5) 76 to 99 percent of cattle vaccinated, and (6) 100 percent of cattle vaccinated.

**Herd Infection Rate**

Infection rates within the six herd classifications are set forth in Table I. Of the total 21,418 herds, 2,349 herds contained reactors. The percent of reactor herds is indicated within each of the six herd classifications. Of the nonvaccinated herds, only 9.48 percent contained reactors. The low infection rate is to be expected in this case, for there has been a common tendency to adopt a vaccination program only when brucellosis has been diagnosed or suspected.

Of the partially vaccinated herds we find the percentage of infected herds highest in the one to 25 percent vaccinated class (16.65 percent). The percentage decreases slightly in the 26 to 50 percent vaccinated class (to 15.60 percent), then drops, but not significantly, in the 51 to 75 percent class (to 15.58 percent). There is a perceptible drop in the percentage of infected herds among the 76 to 99 percent vaccinated class (to 14.26 percent). A highly significant drop (to 7.28 percent) in the percentage of infected herds among the 100 percent vaccinated herds is an indication of a number of known and unknown factors. It is possible that vaccination of all of the cattle in these herds has been instrumental in freeing a few from brucellosis.

Table I includes data on the distribution of herds containing suspects to the brucellosis test, but no reactors, among the various herd classifications.

The percentage of “Suspect Only” herds does not increase in relation to the increase in percentage of vaccinated animals within the herds. A significant drop is noted in the 100 percent vaccinated class. This would seem to indicate that vaccinal titers are not a primary cause for the presence of suspect animals in herds containing only suspects.
TABLE I
Brucellosis as Distributed Through Herd Population in Relation to Herd Vaccination Practices

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonvaccinated</td>
<td>11,102</td>
<td>1,248</td>
<td>807</td>
<td>13,157</td>
</tr>
<tr>
<td>1-25 pct. vacc.</td>
<td>2,025</td>
<td>485</td>
<td>403</td>
<td>2,913</td>
</tr>
<tr>
<td>26-50 pct. vacc.</td>
<td>992</td>
<td>227</td>
<td>236</td>
<td>1,455</td>
</tr>
<tr>
<td>51-75 pct. vacc.</td>
<td>579</td>
<td>131</td>
<td>131</td>
<td>841</td>
</tr>
<tr>
<td>76-99 pct. vacc.</td>
<td>364</td>
<td>73</td>
<td>75</td>
<td>512</td>
</tr>
<tr>
<td>100 pct. vacc.</td>
<td>2,081</td>
<td>185</td>
<td>274</td>
<td>2,540</td>
</tr>
<tr>
<td>Totals</td>
<td>17,143</td>
<td>2,349</td>
<td>1,926</td>
<td>21,418</td>
</tr>
</tbody>
</table>

TABLE I-A

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All partially vaccinated herds</td>
<td>3,960</td>
<td>916</td>
<td>845</td>
<td>5,721</td>
</tr>
<tr>
<td>All nonvaccinated and partially vaccinated herds</td>
<td>15,062</td>
<td>2,164</td>
<td>1,652</td>
<td>18,878</td>
</tr>
</tbody>
</table>

Table I indicates the percentage of all herds found in each of the six classifications. From this information it would seem that there is a tendency toward the vaccination of all calves in the herd if a vaccination program is adopted. This would seem to be a wise course when we consider the decreased infection rate among the 100 percent vaccinated class.

Table I-A contains a portion of the same information as Table I, but it is grouped according to two separate classifications. The first, "All Partially Vaccinated Herds," contains a summary of the herds which fall into the one to 99 percent vaccinated classes. The second, "All Nonvaccinated and Partially Vaccinated Herds" groups all herds except those which were 100 percent vaccinated. The two groupings in Table I-A allow interesting comparisons.

CATTLE INFECTION RATE

Cattle infection rates within each of the six herd classifications are set forth in Table II. Of a total of 951,014 cattle, there were 8,730 reactors (0.92 percent infection rate). The percent of infected animals is indicated within each of the six herd classifications. The 1.06 percent reactors in nonvaccinated herds is a comparatively low infection rate when we consider the fact that this was the first area test in the brucellosis eradication program.

The 1.25 percent animal infection rate in the herds in which one to 25 percent of the animals are vaccinated is highest of all classes as might be
anticipated. As the percent of vaccinated animals in the herd increases, the percent of infected animals decreases until we have only 0.26 percent of the animals infected in the 100 percent vaccinated herds. Some of the 100 percent vaccinated herds have been vaccinating calves for as long as 20 years.

Of interest is the fact that as the percentage of vaccination increases, the percent of suspects decreases. This seems to support the hypothesis that vaccinal titers do not contribute appreciably to the problem of suspects.

**TABLE II**

*Brucellosis as Distributed Through Total Cattle Population in Relation to Herd Vaccination Practices*

<table>
<thead>
<tr>
<th>Herd Vaccination Status</th>
<th>Total Animals No.</th>
<th>Percent</th>
<th>Negative No.</th>
<th>Percent</th>
<th>Reactors No.</th>
<th>Percent</th>
<th>Suspects No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonvaccinated</td>
<td>455,658</td>
<td>47.91</td>
<td>445,320</td>
<td>97.73</td>
<td>4,832</td>
<td>1.06</td>
<td>5,506</td>
<td>1.21</td>
</tr>
<tr>
<td>1-25 pct. vacc.</td>
<td>185,891</td>
<td>19.55</td>
<td>180,110</td>
<td>96.89</td>
<td>2,322</td>
<td>1.25</td>
<td>3,459</td>
<td>1.86</td>
</tr>
<tr>
<td>26-50 pct. vacc.</td>
<td>78,971</td>
<td>8.30</td>
<td>76,917</td>
<td>97.40</td>
<td>707</td>
<td>0.90</td>
<td>1,347</td>
<td>1.70</td>
</tr>
<tr>
<td>51-75 pct. vacc.</td>
<td>46,871</td>
<td>4.93</td>
<td>45,835</td>
<td>97.79</td>
<td>351</td>
<td>0.75</td>
<td>685</td>
<td>1.46</td>
</tr>
<tr>
<td>76-99 pct. vacc.</td>
<td>26,535</td>
<td>2.79</td>
<td>26,123</td>
<td>98.45</td>
<td>110</td>
<td>0.41</td>
<td>302</td>
<td>1.14</td>
</tr>
<tr>
<td>100 pct. vacc.</td>
<td>157,088</td>
<td>16.52</td>
<td>155,475</td>
<td>98.97</td>
<td>408</td>
<td>0.26</td>
<td>1,205</td>
<td>0.77</td>
</tr>
<tr>
<td>Totals</td>
<td>951,014</td>
<td>100</td>
<td>929,780</td>
<td>97.77</td>
<td>8,730</td>
<td>0.92</td>
<td>12,504</td>
<td>1.31</td>
</tr>
</tbody>
</table>

**TABLE II-A**

<table>
<thead>
<tr>
<th>Herd Group</th>
<th>Total Animals No.</th>
<th>Percent</th>
<th>Negative No.</th>
<th>Percent</th>
<th>Reactors No.</th>
<th>Percent</th>
<th>Suspects No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>All partially vaccinated herds</td>
<td>338,268</td>
<td>35.57</td>
<td>328,985</td>
<td>97.26</td>
<td>3,490</td>
<td>1.03</td>
<td>5,793</td>
<td>1.71</td>
</tr>
<tr>
<td>All nonvaccinated and partially vaccinated herds</td>
<td>793,926</td>
<td>83.48</td>
<td>774,305</td>
<td>97.53</td>
<td>8,322</td>
<td>1.05</td>
<td>11,299</td>
<td>1.42</td>
</tr>
</tbody>
</table>

If the number of infected animals can be expected to be about 80 percent less among vaccinated cattle than among nonvaccinated cattle, why is it that we have only an approximate 23 percent reduction in the number of infected herds following prolonged vaccination programs? The answer to this question seems to be conditioned on the fact that the protection offered by vaccination in the face of known exposure is only 60 to 65 percent. The 35 to 40 percent of vaccinated animals which are still susceptible, being evenly distributed throughout the entire herd population, serve to perpetuate the disease among vaccinated populations. They pose a threat of exposure to nonvaccinated contacts as well.

Table II-A contains a portion of the same information as Table II, but it is grouped according to two collective classifications. The first, “All Partially Vaccinated Herds” contains a summary of the cattle which are in the one to 99 percent partially vaccinated herds. The second, “All Nonvaccinated and Partially Vaccinated Herds,” includes all animals other than those in 100 percent vaccinated herds. These two classifications allow interesting comparisons.
The value of any vaccine cannot be determined until the vaccinated animals have been exposed to the specific disease. In order to determine the efficacy of Strain 19 Brucella vaccine under possible exposure conditions in Montana, it was important that comparative studies be made between vaccinated and nonvaccinated populations within known infected herds.

Table III presents the distribution of reactors and suspects between the vaccinated and nonvaccinated populations within partially vaccinated reactor herds. Since exposure opportunity has been equal within each herd, it follows that equal exposure opportunity would apply to both groups collectively. A total of 915 partially vaccinated infected beef herds, containing 84,404 beef cattle, were surveyed on the initial brucellosis test in the Montana area eradication program. At that time, 26.67 percent of the cattle in partially vaccinated herds were vaccinated. Among the nonvaccinated cattle, five percent were reactors. This compares with 1.75 percent among the vaccinated animals. Applying the infection rate of five percent (which would be assumed to be the rate of infection if there were no vaccinations) we would have an expected 1,125 reactors rather than the actual 394. Thus, 731 animals which would probably have been infected were protected from the disease. This is a protection rate of 65 percent in the face of known brucellosis exposure.

Table III-A gives a population summary within the 185 infected herds in the 100 percent vaccinated category. Of the cattle in these herds 1.99 percent were reactors. Applying the infection rate of five percent (derived from the percent of reactors among the nonvaccinated population in partially vaccinated reactor herds), we would have an expected 1,024 reactors rather than the actual 408. Thus, 616 animals that probably would have been infected were protected from the disease. This is a protection rate of 60 percent in the face of known brucellosis exposure. It is surprising to note that the protection rate among vaccinated animals in the 100 percent vaccinated herds is five percent lower than the protection rate among vaccinated animals in the partially vaccinated herds. However, there were a few herds in the 100 percent vaccinated group which were heavily infected. One such herd had 36 reactors and 42 suspects among 248 head of cattle. Apparently the virulence of the organism and exposure opportunity were overwhelming in this particular herd. Such special circumstances must not cause us to reject vaccination. Later tests in this herd show the infection to be almost wiped out through a program of testing, with slaughter of infected animals, and continuing vaccination of replacement calves.

TABLE III
Distribution of Reactors and Suspects Among Vaccinated and Nonvaccinated Populations Within All Partially Vaccinated Reactor Herds

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Total No.</th>
<th>Total Percent</th>
<th>Negative No.</th>
<th>Negative Percent</th>
<th>Suspects No.</th>
<th>Suspects Percent</th>
<th>Reactors No.</th>
<th>Reactors Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonvaccinated</td>
<td>61,896</td>
<td>73.33</td>
<td>55,630</td>
<td>89.88</td>
<td>3,170</td>
<td>5.12</td>
<td>3,096</td>
<td>5.00</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>22,508</td>
<td>26.67</td>
<td>21,532</td>
<td>95.67</td>
<td>582</td>
<td>2.58</td>
<td>394</td>
<td>1.75</td>
</tr>
<tr>
<td>Totals</td>
<td>84,404</td>
<td>100</td>
<td>77,162</td>
<td>91.43</td>
<td>3,752</td>
<td>4.44</td>
<td>3,490</td>
<td>4.13</td>
</tr>
</tbody>
</table>
Table IV gives a detailed breakdown of the brucellosis incidence within vaccinated and nonvaccinated populations, and in all six classifications of herds. The herd classifications are the same as those previously set forth in Tables I and II.

It is significant that there is no appreciable difference in the infection rate among the nonvaccinated populations within the various classifications of herds. In the 76 to 99 percent vaccinated herds, we find an infection rate of 1.16 percent among the nonvaccinated animals. This is greater than the rate in the nonvaccinated herds, and almost as high as the 1.32 percent infection rate among the nonvaccinates in the one to 25 percent vaccinated herds. In other words, there is no appreciable protection for nonvaccinates, regardless of the percentage of vaccinated animals in the herd. This points up the fact that to be effective a vaccination program must include 100 percent of the replacement calves.

In contrast, we note that there is a substantial decline in the infection rate among vaccinated animals as the percentage of vaccinated animals within the herd increases. This would seem to bear out the hypothesis that the higher the percentage of vaccinated animals within a herd, the more effective is the protection afforded each animal vaccinated. However, in the 100 percent vaccinated group we still have an infection rate of a little more than two-fifths that of the rate in vaccinated animals in the one to 25 percent vaccinated herd classification.

### TABLE IV

*Brucellosis as Distributed Through the Nonvaccinated and Vaccinated Cattle Populations in Relation to Herd Vaccination Practices*

<table>
<thead>
<tr>
<th>Herd Vaccination Status</th>
<th>Nonvaccinated Animals</th>
<th>Vaccinated Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Negative-</td>
<td>-Reactors-</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>Nonvaccinated</td>
<td>445,320</td>
<td>97.73</td>
</tr>
<tr>
<td>1-25 pct. vacc.</td>
<td>162,854</td>
<td>96.80</td>
</tr>
<tr>
<td>26-50 pct. vacc.</td>
<td>48,362</td>
<td>96.71</td>
</tr>
<tr>
<td>51-75 pct. vacc.</td>
<td>16,918</td>
<td>96.76</td>
</tr>
<tr>
<td>76-99 pct. vacc.</td>
<td>3,742</td>
<td>96.44</td>
</tr>
<tr>
<td>Totals</td>
<td>677,196</td>
<td>97.40</td>
</tr>
</tbody>
</table>

| Vaccinated Animals      | -Negative- | -Reactors- | -Suspects- | -Total- |
|-------------------------| No.      | Percent | No.      | Percent | No.      | Percent |
| 1-25 pct. vacc.         | 17,256   | 97.70   | 108      | 0.61    | 298      | 1.69    | 17,662   | 6.90    |
| 26-50 pct. vacc.        | 28,555   | 98.59   | 94       | 0.32    | 316      | 1.09    | 28,965   | 11.33   |
| 51-75 pct. vacc.        | 28,917   | 98.40   | 127      | 0.43    | 343      | 1.17    | 29,387   | 11.49   |
| 76-99 pct. vacc.        | 22,381   | 98.79   | 65       | 0.29    | 209      | 0.92    | 22,655   | 8.86    |
| 100 pct. vacc.          | 155,475  | 98.97   | 408      | 0.26    | 1,205    | 0.77    | 157,088  | 61.42   |
| Totals                  | 252,584  | 98.76   | 802      | 0.31    | 2,371    | 0.93    | 255,757  | 100     |
MONTANA BRUCELLOSIS VACCINATION SURVEY

To determine the efficiency of *Brucella abortus* Strain 19 vaccine in beef cattle, a comprehensive survey was conducted in the State of Montana at the time of the first area test for certification. Data on 951,014 cattle in 21,418 herds are presented. The vaccination status of each animal was determined at the time of test. The data are analyzed on the basis of percentage of animals vaccinated within each herd, and comparisons of vaccinated and nonvaccinated populations. The following facts are evident:

1. The herd infection rate was only 23 percent lower in those herds in which all animals were vaccinated than it was in those herds in which none of the cattle were vaccinated.

2. The infection rate within the entire vaccinated cattle population was approximately 80 percent lower than that within the entire nonvaccinated population.

3. Vaccinal titers were not a primary cause for the presence of suspect animals in herds containing suspects, but no reactors.

4. Suspects occurred 36 percent less frequently within the vaccinated population than within the nonvaccinated population.

5. In partially vaccinated infected herds, brucellosis occurred 65 percent less frequently within the vaccinated population than it did within the nonvaccinated population. This, then, is a protection rate of 65 percent in the face of known exposure.

6. In infected herds in which all animals were vaccinated, brucellosis occurred 60 percent less frequently than it did within nonvaccinated cattle in partially vaccinated infected herds.

7. The relative protection afforded by Strain 19 vaccine did not influence the rate of infection within nonvaccinated populations in infected herds, regardless of the percent of animals vaccinated.

8. Vaccination alone cannot be expected to eradicate brucellosis nor to free the majority of infected herds from the disease. Infected animals must be identified and eliminated.

9. Vaccination is very useful in building the resistance of susceptible animals against brucellosis, but its limitations must be recognized.
REPORT OF COMMITTEE ON BRUCELLOSIS


Mr. President, Members of the Association, and Invited Guests: As it has been previously reported by this Committee, the present program of brucellosis eradication with amendments was officially approved by this Association and the then Bureau of Animal Industry, United States Department of Agriculture in Chicago in 1947. At that time there was but one state which had qualified as a modified certified brucellosis area, namely, North Carolina. In 1949, New Hampshire joined the modified certified list, to be followed two years later by the State of Maine. While the program was carried on by the several states in the years that followed, it was not until 1956 that two more states joined the select list, namely, Wisconsin and Washington. The following year, 1957, the States of Minnesota, Delaware, Connecticut and Vermont received their certified brucellosis certificates, making a total of nine states. Now, just one year later, we find that on September 30, 1959, there are 1,718 counties, 21 states, Puerto Rico, and the Virgin Islands listed in the modified certified brucellosis areas. In addition to this, there were 401 counties that received complete area testing, and 1,033 counties where individual herd participation was being carried on.

The records show that definite and satisfactory progress has been made in recent years, and all indications are to the effect that the remaining states will meet their goal during the next few years provided sufficient funds are available. While the prime object might be to test all animals within a given area and reduce the infection to the percentage required to qualify as a modified certified brucellosis area, we must not neglect or lose sight of the fact that continued testing and eternal vigilance be exercised in all of the states and areas which have already qualified if we are to hold the line and advance our fronts in new areas.

The price of continued freedom from the returning source of bovine brucellosis, which has been practically eradicated from so many of our states and is well under control in many others, as well as the pressing need to wage increased warfare against other dangerous and economic live stock diseases, demands of us constant watchfulness and service. In the years since the United States Livestock Sanitary Association has been in existence, many
men and women have contributed to the control and eradication of the many costly diseases that our domestic animals are heir to, and in the spirit of those who have fought and fallen, and who have left to us the slogan, "Carry On," we must not fail, we offer this tribute:

"They spoke it bravely, grimly, in times of fear and doubt;  
They spoke it when the tide of faith and hope seemed ebbing out;  
But they buckled on the armor in the troubled days now gone  
And left to us their slogan, 'Comrades, Carry On.'  
'Carry On,' when critics scoff and scorn you, 'Carry On' and doubt despair.  
'Carry On,' you'll win the battle though the burden's hard to bear.  
'Twas the slogan that they gave us as they fell beside the way  
And for them and those who follow, we must 'Carry On' today."

Last year at Miami Beach, Florida, your Committee, after holding several hearings re-wrote the uniform rules and regulations governing the eradication of bovine brucellosis. These rules and regulations were approved by the Agricultural Research Service in February, 1959, and printed in pamphlet form. However, no program, no matter how well organized it may be, is so perfect that it will not need amendments and changes from year to year. With the untiring work of individuals engaged in scientific endeavor and research, new tests and new methods of procedure must be adopted from time to time, if and when such tests and procedures have been proven beyond the shadow of a doubt that they are sound, workable and can help materially in the control and eradication of diseases in our domestic animals, then and only then should these tests be accepted and incorporated as a part of our uniform rules and regulations.

During the past year there have been a number of suggestions made to your Committee relative to the certification of range and semi-range areas. In February at the annual meeting of the National Brucellosis Committee, which your chairman attended, this question received a great deal of attention with the result that at a meeting of the regulatory officials of the North Central States held in Sioux Falls, South Dakota in March, your Chairman, together with the Chairman of the National Brucellosis Committee, Dr. Sam McNutt; Thomas Arnold, Chairman of the Fact Finding Committee of the American National Cattlemen's Association; Dr. C. K. Mingle of the Agricultural Research Service, and others, discussed the program extensively with the regulatory officials of the North Central States. In April, at Denver, Colorado, a similar meeting was held and the same subject was discussed with members of Mr. Arnold's committee together with ranchers of the range states. This meeting was adjourned to November 2, 1959, when at the request of Mr. Arnold and his committee, Doctors Anderson and Mingle of the Agricultural Research Service, United States Department of Agriculture, together with Dr. Sam McNutt, Chairman of the National Brucellosis Committee, Mr. William Knox, Editor of Hoard's Dairyman and member of the Brucellosis Committee of the United States Livestock Sanitary Association, and your chairman, discussed at length the brucellosis eradication program as it affected range and semi-range states and those states who purchase large numbers of feeder cattle from the range states. As a result of these meetings, a resolution spon-
sored by a Committee of the American National Cattlemen's Association, together with similar resolutions from other interested states and individuals were filed with your Committee for consideration. In last year's report Part V, providing for a certified brucellosis-free area, was added to our uniform rules and regulations. However, specific requirements were not spelled out, nor were rules and regulations adopted to provide for the recertification of a free area once it had been established. All of the resolutions and proposed amendments to the uniform rules and regulations have been given extensive and exhaustive hearings by your Committee and we are now ready to submit to you our recommendations:

1. Your Committee reviewed ADE Division Memorandum 504.21 as amended and believes the outline of procedure will assist in carrying out the Brucellosis Eradication Program as set forth by the Uniform Methods and Rules.

2. Your Committee received a request to permit recertification of areas on the basis of official calfhood vaccination alone. Conclusive evidence must be presented, proving the safety of certification in this manner. Since such evidence was not presented, nor is it available so far as the committee is able to learn, the request was tabled.

3. Part V of the Uniform Methods and Rules has been expanded to set forth requirements to obtain and maintain certified areas.

4. Several additional amendments have been made to the Uniform Methods and Rules, therefore, there is attached the complete rewrite of the rules.

BOVINE BRUCELLOSIS ERADICATION
UNIFORM METHODS AND RULES

PART I: DEFINITIONS

"Positive" or "Reactor"

1. An official vaccinate more than thirty (30) months of age that discloses a complete agglutination reaction in the blood titer dilution of 1/200 or higher.

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

"Suspect"

1. An official vaccinate more than thirty (30) months of age that discloses agglutination in the 1/100 dilution and less than complete in the 1/200 dilution.

2. All other animals more than six (6) months of age that disclose agglutination in the 1/50 dilution and less than complete agglutination in the 1/100 dilution.
"Negative"
1. An official vaccinate more than thirty (30) months of age that discloses a reaction of not more than complete agglutination in the 1/50 dilution.
2. All other animals more than six (6) months of age that disclose a reaction of less than incomplete agglutination in the 1/50 dilution.

Note: Animals which at the time of vaccination exceed the maximum ages or have not attained the minimum age specified under the definition of an "official vaccinate" have no status as vaccinated animals. (Plan "D" is no longer a part of the Uniform Methods and Rules.)

The "Herd" Test

Shall include all cattle over eight (8) months of age except steers, spayed heifers and official vaccinates not more than thirty (30) months of age.

"Approved Brucella Vaccine"

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

"Official Vaccinate"

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

Identification of Vaccinated Animals

(a) Calves tattooed "shield and V" in right ear or branded "V" on right jaw.

(b) If the tattoo is used, then the "shield and V" shall be preceded by a numeral indicating the quarter of the year in which the vaccination was done. The "shield and V" shall be followed by the last digit of the year in which the vaccination was done.

(c) If the brand is used, then the "V" shall be applied in four different positions—one each year over a four-year period to indicate in which year the vaccination was done. The fifth year will repeat the first year, and so on indefinitely. In 1960 the "V" shall be placed with the open end facing downwards and so on clockwise indefinitely.

"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 Sanitary Authority local factors prevail which are not conducive to the eradication of brucellosis, such areas need not be classed as range or semi-range.

“Immediate slaughter”

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

**PART II. RECOMMENDED PROCEDURES**

Section I. *Individual Herd Plans*

*Plan A.* Testing of cattle, permanent identification, and prompt disposal of positives, for slaughter only, with or without vaccination of calves. Herds that have passed three successive satisfactory milk ring tests at intervals of not less than four (4) nor more than six (6) months, may be considered as having met the brucellosis requirements of Plan A for Grade A milk production.

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

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

Section II. *Participation on Area Basis*

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

B. When an area has been designated as working toward Modified Certified Brucellosis Area status, as a result of petitioning or other legal procedure, all herds and cattle shall be placed under the provisions of the Modified Certified Brucellosis Area plan as outlined in Part IV.
BRUCELLOSIS

C. When at least 90 percent of the counties representing a majority of the cattle in the state have been legally designated as working toward Modified Certified Brucellosis Area status, the remaining herds and cattle in the state shall be placed under the Modified Certified Brucellosis Area plan.

Section III. Supervision

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

Section IV. Entering Premises

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

Section V. Services to Owner

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

Section VI. Classification of Animals

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

<table>
<thead>
<tr>
<th></th>
<th>1/50</th>
<th>1/100</th>
<th>1/200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official Vaccinates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Suspect</td>
<td>+</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>Positive</td>
<td>+</td>
<td>+ I</td>
<td>+</td>
</tr>
</tbody>
</table>

Section VII. Branding Positives

A permanent brand with the letter “B” (at least 2x2 inches) must be placed on the left jaw of all cattle classed positive. Positives must remain on premises where disclosed until a state or federal permit has been obtained for movement to immediate slaughter where state approved and/or federal inspection is maintained.

Section VIII. Reports

All activities, conducted either privately or as part of the official program such as results of agglutination tests and vaccination, must be reported promptly to State and Federal cooperating agencies.
PART III: INDIVIDUAL CERTIFIED HERD PLAN

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

Section I. Herd Certification

A. *Herd blood tests* shall be made at intervals of not more than sixty (60) days until all evidence of infection has been eliminated. A herd may be certified as brucellosis-free when it has passed at least two consecutive negative blood tests, with the first clean test and the certifying test not less than twelve (12) months nor more than eighteen (18) months apart. Additional herd tests may be conducted on herds if the owner so desires, or if the certifying agency deems it advisable.

B. If there is no evidence of infection on the first blood test, a herd may be certified as brucellosis-free when it has passed one additional negative test conducted not earlier than six (6) months nor more than eighteen (18) months from the date of the first test.

C. Where the Milk Ring Test is employed, herds may be initially certified as brucellosis-free with a minimum of three satisfactory milk tests conducted at not less than ninety (90) day intervals and followed by a negative herd blood test conducted within ninety (90) days after the last negative milk ring test. Herds in which infection has been disclosed shall be tested in accordance with provisions of paragraph A of this section.

Section II. Herd Recertification

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

B. If the retest of a certified herd or of animals from such a herd reveals one reactor, the herd may be recertified on the result of a negative herd retest conducted not earlier than sixty (60) days following removal of the reactor. Intervening blood tests, if any, must be negative.

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

D. If the retest of a certified herd discloses suspects but no positives, only the suspicious animals need be retested. If all suspects are available for retest and are negative, the herd test may then be considered negative. If the retest does not include all suspicious animals, or if one or more positives results, the herd shall be retested as provided in Section II, B or C. If only one suspect was not available for retesting and if that suspect had been an official vaccinate, the herd test shall be considered negative and the herd recertified.
Section III. General Provision

A. Vaccination Titers. Official vaccinates under thirty (30) months of age are not required to be tested, or if tested, are not required to be negative. Official vaccinates over thirty (30) months of age, classed suspect, may be retained in certified herds for retesting until their final determination is made.

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

1. To certified herds:
   a. Natural herd increase.
   b. From herds with equal status.
   c. From herds that have passed a negative blood test within the past twelve (12) months:
      (1) Official vaccinates under thirty (30) months of age on certificate of vaccination—over thirty (30) months of age if negative within thirty (30) days prior to addition.
      (2) Non-vaccinated animals on evidence of negative retest not less than sixty (60) days from date of negative herd test.

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

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

D. Certificates. Certified Brucellosis-free herd certificates which shall be valid for one year unless revoked, may be issued by cooperating state or federal officials.

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

F. Cleaning and disinfection. Premises shall be cleaned and disinfected under regulatory supervision within fifteen (15) days following removal of reactors, unless an extension of time is granted.

PART IV: MODIFIED CERTIFIED AREA PLAN

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

Section I. Area Certification

A. If as the result of a blood test of all cattle within an area the number of positives does not exceed one percent and the herd infection does not exceed five percent, the area may be declared a Modified Certified Brucellosis Area for a period of three (3) years. Infected herds shall be quarantined until they have passed one negative blood test at least thirty (30) days following removal of the cattle classed positive except cattle consigned for immediate slaughter under permit.

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

C. (1) Range and semi-range areas may qualify as Modified Certified Brucellosis Areas for a period of three years if as the result of a blood test of all dairy cattle, all purebred cattle, and not less than 20 percent of the range and semi-range cows over three years of age in each herd, the number of positive does not exceed one percent of the area cattle population (excluding steers and spayed heifers) and five percent of the herds. Two or more semi-annual milk ring tests with blood tests of suspicious herds may be substituted for blood tests of individual dairy herds.

(2) Should evidence of infection be disclosed in any of the animals required to be tested in the range or semi-range herds under provisions of Part IV, Section I, C. (1), such herds shall be quarantined until (a) the entire herd has passed one negative blood test at least thirty (30) days fol-
following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit, or (b) all cattle eligible for test that are sold or removed from the herd have been tested and no reactors are found following the removal of all cattle classed positive, provided a sufficient number of cattle have been tested to conform with ARS graph, October 1959, and provided further, that all replacement heifer calves have been officially vaccinated during the quarantine period. Those herds which do not have consecutive negative blood test on sufficient cattle to equal the minimum sample size during the four-year period immediately following removal of the reactors will be handled in accordance with the provisions of C (2) (a).

D. If testing as outlined under Part IV, Section I, Paragraphs A, B, or C reveals an animal infection rate of more than one percent but not over two percent, and/or a herd infection rate of more than five percent, and an initial retest of a sufficient number of the infected herds applied not less than thirty (30) nor more than one hundred twenty (120) days following removal of the cattle classed positive reduces infection rates to not more than one percent animal infection and not over five percent herd infection, the area may then be certified. All infected herds shall be quarantined until the entire herd has passed one negative blood test at least thirty (30) days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit.

E. If the test of an area as outlined under Part IV, Section I, Paragraphs A, B, or C results in more than two percent positives, or if an initial retest of infected herds as under Section I, Paragraph D, does not qualify the area for certification, it shall be necessary to make a complete area retest.

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.

Section II. Area Recertification

A. At the expiration of the three-year period areas certified under the provision of Part IV, Section I, Paragraphs A or B, may be recertified for another three-year period. To do so, the results of a test of all herds in which infection was reported at the time of the previous certifying test or since, together with the results of a test of at least 20 percent of other representative herds, must reflect a rate or infection which does not exceed one percent of the cattle and five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same herds previously tested for this same purpose.

B. Areas certified under the provisions of Part IV, Section I, Paragraphs A or B, may be continued as certified with the application of semi-annual milk ring tests, follow-up blood tests of milk suspicious herds, and blood tests at three-year intervals on at least 20 percent of all herds not included in the milk test and herds in which the BRT does not represent a majority
of the cattle in the herds, or a screen test of beef-type herds in accordance with ADE Division Memorandum No. 504.21, as amended if the incidence of infection does not exceed one percent of the cattle, and five percent of the herds. The herds tested to meet the 20 percent requirement shall not include the same herds previously tested for this purpose.

C. (1) At the expiration of the three-year period, range and semi-range areas may be certified for another three-year period when at least 20 percent of the herds, including animals as outlined under Part IV, Section I, C (1) have been retested and the animal infection rate does not exceed one percent in not more than five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same group previously tested for this same purpose.

(2) Modified Certified Brucellosis Range or Semi-range Areas may be maintained in a certified status for additional periods of three years, provided:

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

(b) That during each year at least five (5) percent of the breeding cows in the area, as determined by the statistics of the Agricultural Marketing Service, or a total of fifteen (15) percent during a three-year period, are subjected to the agglutination test for brucellosis; the blood samples to be taken from animals at farms, ranches, sale yards, or slaughtering establishments.

(c) That herds in which the existence of brucellosis has been established by "cull and slaughter testing" are blood tested and handled according to the provisions of Part IV, Section I, C (2).

(d) That dairy herds in the area are screened semi-annually by the milk ring test, with blood test of herds suspicious to the milk ring test, or are blood tested.

(e) That purebred beef herds are blood tested or that during the certification period at least 15 percent of the breeding cows in the herd are subjected to the agglutination test for brucellosis; the blood samples to be taken from cull and slaughter cows at ranches, sale yards, or slaughtering establishments. If less than 15 percent of the breeding cows in the herd have been tested among the cull and dry cows, sufficient additional cows may be tested within the herd to bring the total to at least 20 percent of the breeding cows over three years of age.

(f) That the percentage of infection disclosed as a result of such tests as conducted under the provisions of the above four paragraphs does not exceed five percent of the herds, and one percent of the area cattle population over six months of age (excluding steers and spayed heifers); the number of reactors used in computing the percentage to be the number accumulated over the three-year period.
D. If testing as outlined under Part IV, Section II, A, B, C (1) or C (2) reveals an animal infection rate of more than one percent but not over two percent, and/or a herd infection rate of more than five percent, and an initial retest of a sufficient number of the infected herds applied not less than thirty (30) nor more than one hundred twenty (120) days following removal of the cattle classed as positive reduces infection rates to not more than one percent animal infection and not over five percent herd infection, the area may then be recertified. All infected herds shall be quarantined until the entire herd has passed a negative blood test at least thirty (30) days following removal of the cattle classed positive except cattle consigned for immediate slaughter.

E. Any area not qualifying for recertification under the provisions of Part IV, Section II shall be required to reestablish its certified status through testing procedures as outlined under Part IV, Section I.

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.

Section III. Additions to Modified Certified Brucellosis Areas

A. Cattle from officially Certified Brucellosis-Free herds or Areas and cattle from negative herds in Modified Certified Brucellosis Areas may enter Modified Certified Brucellosis Areas without being retested for brucellosis. All such cattle shall be individually identified and shall be accompanied by an approved certificate of health indicating herd and animal status.

B. Cattle from herds under federal-state supervision for the control of brucellosis may enter a Modified Certified Brucellosis Area or an area in the process of such certification when all animals in the herd were negative to the official blood agglutination test for brucellosis within 90 days of the date of entry. Individual animals to be moved must be negative to an official retest at least 30 days from the date of the previous herd test and within 30 days of entry or be official vaccinates under 30 months of age.

C. Cattle under 30 months of age officially vaccinated and coming from (a) negative herds in Modified Certified Brucellosis Areas, (b) Certified Brucellosis-Free herds or Areas, or (c) herds under federal-state supervision which have passed a test as under paragraph B may enter a Modified Certified Brucellosis Area or an area in the process of certification without further test when individually identified by mark, brand, tattoo or other acceptable identification, and accompanied by a certificate of health approved by the proper sanitary official of the state of origin.

D. Breeding cattle not over 30 months of age, officially vaccinated, which do not qualify under paragraph C may enter a Modified Certified Brucellosis Area providing they do not show blood agglutination reactions higher than incomplete in dilution of 1/200 and the animals are maintained in quarantine until they have passed a negative retest.

E. All other male or female cattle over eight months of age, including official vaccinates over thirty (30) months of age, except steers, spayed
heifers, and cattle intended for immediate slaughter, shall be required to pass a negative officially recognized blood agglutination test for brucellosis within 30 days prior to the date of entry. They shall be maintained in quarantine separate and apart from all other cattle and be retested in not less than 30 nor more than 90 days after date of entry. If retested and classed negative, they shall be released from quarantine.

PART V: CERTIFIED BRUCELLOSIS-FREE AREAS

The provisions of the individual certified herd plan that relate to testing, quarantining, cleaning and disinfecting shall apply to Certified Brucellosis-Free areas.

Section I. Establishing Certified Brucellosis-Free Areas

Areas may qualify as Certified Brucellosis-Free for a period of five years provided:

1. They are located in a state in which all areas have current Modified Certified Brucellosis Area status or Certified Brucellosis-Free Area status.
2. All herds in the area have been included in one of the following within 18 months immediately preceding the request for Certified Brucellosis-Free Area status:
   (a) at least three semi-annual brucellosis ring tests.
   (b) the cull and dry cow testing program, under which the equivalent of at least five percent of the breeding cows in the herd over three years of age are blood tested each year.
   (c) a blood test of a sample group of the breeding cows over three years of age, the sample to contain enough animals to provide a 95 percent probability of finding brucellosis, if present, at the two percent level of disease incidence among the animals tested.
   (d) a complete herd blood test.
3. All brucellosis ring test suspicious herds have been blood tested.
4. Not more than one percent of the herds, or one herd, whichever is greater, shall have been found to be infected during the 18 months immediately preceding the request for Certified Brucellosis-Free Area status.
5. Not more than 0.2 percent of the cattle shall have been found to be reactors during the 18 months immediately preceding the request for Certified Brucellosis-Free Area status.
6. All suspects to the blood serum test shall:
   (a) have been retested negative,
   (b) have had at least two retests not less than 30 days apart in which it has been established that the blood serum titers are stabilized or receding,
   (c) be a part of a herd included under 2 (a) or (b) of this section, or
(d) if the suspects have been consigned to slaughter and are not available for retest, the herd shall be retested or be included under 2 (a) or (b) of this section.

7. All herds in which brucellosis has been known to exist have been legally released from quarantine.

8. All herds in which brucellosis has been found during the latest certification period shall have been retested not less than three months following removal of the last reactors, or be a herd included under 2 (a) or (b) of this section.

9. A system employing positive identification of all slaughter cows and bulls over 30 months of age, whether of beef or dairy breeds, moving in intrastate and interstate commerce, shall have been initiated in the area.

10. All herds of other species of domestic livestock in which brucellosis has been found or suspected have either been tested negative or eliminated, leaving no known foci of infection.

Section II. Maintaining Certified Brucellosis-Free Areas

A. The period of certification may be extended from five to six years provided at least 80 percent of the eligible calves retained in the area each year are officially vaccinated.

B. Certified Brucelosis-Free Areas may be recertified at the end of each period of certification provided:

1. All herds in the area are represented in one of the following:
   (a) brucellosis ring tests conducted at least semi-annually.
   (b) blood tests of cows and bulls over 30 months of age consigned to market for breeding, feeding, slaughter or other purposes, whether of dairy or beef breeds. The number of animals tested each year must be at least five percent of the animals over three years of age in the herd (25 percent over a five-year period or 30 percent over a six-year period).
   (c) A blood test of a sample groups of the breeding cows over three years of age, the sample to contain enough animals to provide a 95 percent probability of finding brucellosis, is present, at the two percent level of disease incidence among the animals tested. The blood test is to be conducted within 18 months prior to the termination of the certification period.
   (d) A complete herd blood test conducted within 18 months prior to the termination of the certification period.

2. All brucellosis ring test suspicious herds have been blood tested.

3. All herds included in 1 (b) or (c) in which it has been established that brucellosis exists are blood tested.

4. The number of herds found infected during the entire certification period does not exceed one percent of the area herd population, or one herd, whichever is greater.
5. The number of animals found infected during the entire certification period does not exceed 0.2 percent of the area cattle population over 30 months of age. For purposes of this calculation, of animals consigned to market, only those which were tested within 14 days of the time they left the premises of the original owner are to be included.

6. Herds in which brucellosis has been found have been retested and legally released from quarantine, and, in addition, have been retested not less than three-months following removal of the last reactors or be a herd included under 1 (a) or (b) of paragraph B, this section.

7. All suspects to the blood serum test shall:
   (a) have been retested negative,
   (b) have had at least two retests not less than 30 days apart in which it has been established that the blood serum titers are stabilized or receding,
   (c) be a part of a herd included under 1 (a) or (b) of paragraph B, this section, or
   (d) if the suspects have been consigned to slaughter and are not available for retest, the herd shall be retested or be included under 1 (a) or (b) of paragraph B, this section.

8. If any reactors are disclosed in Certified Brucellosis-Free Areas, they are to be consigned to slaughter. If they are to be marketed within a Certified Brucellosis-Free Area, they should be transported directly to the holding pens of a slaughtering establishment. In any event, they are not to be unloaded onto premises or into facilities within a Certified Brucellosis-Free Area, which are utilized for holding or handling livestock for any other purpose than consignment for slaughter. Vehicles used for transporting reactors must be cleaned and disinfected under the supervision of a federal or state inspector or an accredited veterinarian before again being used for transporting any other livestock.

9. All herds of other species of domestic livestock in which brucellosis is suspected or found shall be placed under quarantine and retested until negative or be eliminated.

C. If the percentage of infected herds or reactors exceeds the percentages set forth in B 4 and 5, the area shall revert to Modified Certified Brucellosis Area status and must requalify in accordance with Section I of Part V.

Section III. Additions to Certified Brucellosis-Free Areas

A. To enter a Certified Brucellosis-Free Area, cattle (except steers, spayed heifers and calves under 8 months of age) must be from one of the following:

1. Certified Brucellosis-Free Herd.
2. Certified Brucellosis-Free Area.
3. Herd not under quarantine in a Modified Certified Brucellosis Area, and the individual animals blood tested negative within 30 days prior to entering the area. The 30-day test is not required if the herd is actively participating in a screen test procedure.
4. Herd not under quarantine in a Modified Certified Brucellosis Area, and be an official vaccinate under 30 months of age.

B. Animals not qualifying under paragraph A may enter a Certified Brucellosis-Free Area provided they have been held in isolation on premises not known to be infected with brucellosis, and have, while in isolation, passed two negative blood tests, not less than 30 days part, the second to be within 30 days of entering the area. Should any reactors be disclosed in a group of cattle held in isolation under this paragraph none of the animals in the group will be eligible for entry into a Certified Brucellosis-Free Area, regardless of individual animal tests.

C. All animals entering a Certified Brucellosis-Free Area shall be transported in conveyances which have been cleaned and disinfected under the supervision of a federal or state inspector or an accredited veterinarian since last used for hauling livestock.

D. Animals not known to be infected with *Brucella* and those known to be reactors may be consigned to slaughter at establishments within a Certified Brucellosis-Free Area provided they are transported directly to holding pens at the slaughter establishment and are not unloaded onto premises or into facilities utilized for holding or handling livestock for any other purpose than consignment for salughter.
A PRELIMINARY REPORT ON A COMPARISON OF AGGLUTINATION-LYSIS, CAPILLARY TUBE, AND HEMOLYTIC TESTS FOR THE DIAGNOSIS OF BOVINE LEPTOSPIROSIS

LEIF RINGEN *

One of the problems of the diagnosis of bovine leptospirosis is the detection of the actively infected animal. The usual procedures rely on serology and many of the serological tests are serotype specific, thus requiring a battery of antigens. Furthermore, no standardized procedure has been established, either as to which test, or what serum dilutions should be used. It is difficult to determine what would be considered a significant titer, since high serum antibody titers do not necessarily reflect severity of infection.

The tests most commonly used in diagnosis are the agglutination-lysis (AL) (1), and the capillary tube (CT) (2) tests. The AL is the most sensitive, but also has a limitation in diagnosis in that the titer remains at a diagnostic level for years. The CT antigen is a killed stable product, but the test is serotype specific and the antigen prepared from only one serotype.

A genus specific antigen has been described and utilized in a serologic test now known as the hemolytic (HL) test (3, 4). This antigen is stable and easily obtained from Leptospira biflexa, thus eliminating the handling of pathogenic serotypes. Since it is not serotype specific, one antigen will detect the presence of antibodies against any of the serotypes.

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The following report is a preliminary study of the HL test as compared with the AL and CT tests for the diagnosis of bovine leptospirosis.

MATERIALS AND METHODS

A. The Hemolytic Test: The procedure for the hemolytic test was essentially the same as that described by Cox (4), except 4-fold serum dilutions were made ranging from 1:10 to 1:160.

The HL antigen was obtained by growing Leptospira biflexa in a phosphate buffered serum (10 percent) water for two weeks. Cells harvested by centrifugation at 10,000 RPM for 30 minutes were resuspended to 0.1 original volume in a 50 percent ethanol solution containing 0.85 percent NaCl and held in the water bath at 60-70° C for four hours; after overnight refrigeration the suspension was again centrifuged at 10,000 RPM for 30 minutes and the supernatant brought to 90 percent by volume with ethanol, and placed in

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A PRELIMINARY REPORT OF BOVINE LEPTOSPIROSIS

the freezer for six days. The precipitate formed was obtained by centrifugation and dissolved in Mayer's Buffer \(^{5}\), to 0.025 original volume.

Sheep erythrocytes (r.b.c.) were collected in an equal volume of modified Alsever's solution, washed six times in a dextrose-gelatin-veronal buffer (DGV), then suspended in the DGV so as to have a 10 percent suspension.

Complement (Difco Laboratories or Delta Biochemicals Inc.) was reconstituted according to directions, divided into one ml aliquots and frozen.

B. The Agglutination-lysis Test: The Al test was carried out using \(L.\) pomona as the antigen and employing 10-fold serum dilutions ranging from \(10^{-1}\) to \(10^{-3}\). The tests were read by darkfield microscopy.

C. The Capillary Tube Test: The procedure for the CT test was that described by Stoenner \(^{2}\).

D. Bovine Sera: Serum samples were collected from both experimentally infected cattle and field cases. Animals were experimentally infected with \(Leptospira\) pomona and serum collected at periodic intervals. The field cases were serum samples sent to our laboratory for diagnosis of leptospirosis. For the hemolytic test the samples were diluted 1:10, inactivated at 58° C for 30 minutes and adsorbed with 0.1 volume 10 percent r.b.c. just prior to use.

E. Leptospiruria: Leptospiruria was determined in the experimentally infected animals by laboratory animal inoculation \(^{6}\).

RESULTS AND DISCUSSION

A. Sera from experimentally infected animals

The following results were obtained from 30 animals experimentally infected with \(L.\) pomona (Figure 1). These serum samples were collected at intervals over a period of 20 weeks. In order to have weekly tests on each animal two assumptions were made: (1) Serum samples, if collected between two positive tests, would be considered as positive, and (2) once an animal had shown a negative result, it would remain negative on any subsequent testing.

All animals became positive to the Al test within two weeks post exposure and remained positive throughout the experimental period. Although the majority of these animals were positive to the CT and HL at two weeks, at least four weeks were required for all of the animals to become positive to the CT test. Eight percent of the animals tested never developed a detectable hemolytic antibody titer, nor was it possible to demonstrate leptospiruria in these animals.

The number of animals shedding leptospires in their urine gradually increased to reach a maximum of 70 percent which occurred at seven weeks post exposure. This was followed by a rapid decline with no animals showing leptospiruria beyond the thirteenth week. The duration of leptospiruria varies with individual animals, but not all animals that became infected developed a demonstrable leptospiruria.

Several of the animals became negative to the CT as early as eight weeks post exposure, and an increase in number of negative results were obtained so that only 25 percent of the animals were still showing positive reactions.
A comparison of the agglutination-lysis, capillary tube, and hemolytic tests with leptospiruria in a group of experimentally infected cattle.

At the end of the experimental period, animals were becoming negative to the HL test as early as three weeks post-exposure and all the animals were negative at the end of 20 weeks. Leptospiruria could not be demonstrated in those animals which became negative to the serologic tests.

Comparisons were made of the reactions obtained in the various serum dilutions in an attempt to establish significant titers. Since the AL test probably is the most sensitive of the three, it was used as the basis for the comparisons. The AL titer was 1:100 or higher by the end of two weeks post-exposure, so this was considered as indicative of infection. On a comparative basis, it was then found that equivalent titers equaled a 2+ reaction at a serum dilution of 1:160 in the CT test, and a 4+ reaction at a 1:10 dilution for the HL test. Anything less than this was considered as suspect or negative.

B. Sera from field cases

The following results were based on 479 serum samples sent to our laboratory for diagnosis of bovine leptospirosis. The serum samples were classified as positive, suspect, and as negative reactions, the dilution for each being based upon the results obtained from the experimentally infected animals.
TABLE 1

Results Obtained When Testing Bovine Serum Samples by Three Different Serological Tests

<table>
<thead>
<tr>
<th></th>
<th>AL 1</th>
<th></th>
<th>HL 2</th>
<th></th>
<th>CT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Animals</td>
<td>Percent</td>
<td>No. Animals</td>
<td>Percent</td>
<td>No. Animals</td>
</tr>
<tr>
<td>Positives</td>
<td>201/479</td>
<td>42</td>
<td>73/479</td>
<td>15</td>
<td>146/479</td>
</tr>
<tr>
<td>Suspects</td>
<td>75/479</td>
<td>16</td>
<td>27/479</td>
<td>6</td>
<td>37/479</td>
</tr>
<tr>
<td>Negatives</td>
<td>203/479</td>
<td>42</td>
<td>379/479</td>
<td>79</td>
<td>296/479</td>
</tr>
</tbody>
</table>

1 Agglutination-lysis test.
2 Hemolytic test.
3 Capillary tube test.
4 Positive titers were considered as follows: AL-1:100 or higher; HL-1:10 or higher; CT-1:160 or higher.
5 Numerator—number of sera showing reaction.
   Denominator—total number of serum samples tested.
6 Suspect reactions were considered as follows: AL-1:10; HL-less than 4+ at 1:10; CT-1:40.

As would be expected the greatest number of positive and suspect reactions occurred with the AL test, and the greatest number of negative reactions with the HL test (Table 1). Undoubtedly, many of the serum samples were collected after the infection had been established in a herd, and after many of the animals had passed the actively infected state. Table 2 shows how the various serum samples which were considered as positive, suspect and negative to the AL test reacted to the other two tests. Table 3 shows the same results for the CT test and Table 4 for the HL test. The results indicate that if the sample is positive to the HL test it will also show a reaction, either positive or suspect, in the other two tests; if it is positive to the CT it will also show reaction to the AL, but not necessarily to the HL. The same sequence was found in those samples considered as suspects. However, in those samples showing negative results the opposite was found; that is, if they were negative to the AL they would also be negative to the other two; if they were negative to the CT they would also be negative to the HL, but not necessarily to the AL. At first, no attempt was made for the standardization of the HL antigen except to include known positive and known negative bovine serum when setting up the tests. However, the same serum samples were retested with different batches of antigen with reproducible results. Later the antigen was standardized by the procedure described by Cox (4).


## TABLE 2

*Comparison of the Hemolytic and Capillary Tube Test to the Agglutination-lysis Test*

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Percent</th>
<th>Suspect</th>
<th>Percent</th>
<th>Negative</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AL(^1)</td>
<td>201</td>
<td>75</td>
<td></td>
<td></td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>AL-HL-CT(^2)</td>
<td>69</td>
<td>34</td>
<td>1</td>
<td>1</td>
<td>176</td>
<td>87</td>
</tr>
<tr>
<td>AL(^3)</td>
<td>47</td>
<td>23</td>
<td>64</td>
<td>85</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>AL-HL(^4)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>AL-CT(^5)</td>
<td>81</td>
<td>40</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^1\) Results obtained from 479 serum samples tested by the AL test.
\(^2\) Number of AL serum samples showing agreement in other two tests.
\(^3\) Number of AL serum samples not showing agreement with other two tests.
\(^4\) Number of serum samples showing agreement with the HL test.
\(^5\) Number of serum samples showing agreement with the CT test.

Based upon the results from the experimentally infected animals, high blood serum antibody titers do not necessarily reflect the severity of the infection. Therefore, diagnostic procedures based upon serology could use relatively few serum dilutions, providing the antigen were standardized properly. Currently, experiments are being carried out in an attempt to determine the number of units of HL antigen most suitable. Since the serum antibody titer obtained is dependent upon the concentration of antigen, attempts are being made to adjust the antigen concentration so that a 4+ reaction at a serum dilution of 1:10 would indicate either an actively or recently infected animal.

## TABLE 3

*Comparison of the Agglutination-lysis and Hemolytic Tests to the Capillary Tube Test*

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Percent</th>
<th>Suspect</th>
<th>Percent</th>
<th>Negative</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CT(^1)</td>
<td>146</td>
<td>37</td>
<td></td>
<td></td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>CT-AL-HL(^2)</td>
<td>63</td>
<td>43</td>
<td>1</td>
<td>3</td>
<td>176</td>
<td>60</td>
</tr>
<tr>
<td>CT(^3)</td>
<td>4</td>
<td>3</td>
<td>25</td>
<td>68</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CT-AL(^4)</td>
<td>72</td>
<td>49</td>
<td>7</td>
<td>19</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CT-HL(^5)</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>104</td>
<td>35</td>
</tr>
</tbody>
</table>

\(^1\) Results obtained from 479 serum samples tested by the CT test.
\(^2\) Number of CT serum samples showing agreement in other two tests.
\(^3\) Number of CT serum samples not showing agreement in other two tests.
\(^4\) Number of serum samples showing agreement with the AL test.
\(^5\) Number of serum samples showing agreement with the HL test.
TABLE 4
Comparison of the Agglutination-lysis and Capillary Tube Tests to the Hemolytic Test

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Percent</th>
<th>Suspect</th>
<th>Percent</th>
<th>Negative</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HL1</td>
<td>73</td>
<td>17</td>
<td>379</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-AL-CT2</td>
<td>57</td>
<td>78</td>
<td>1</td>
<td>4</td>
<td>176</td>
<td>47</td>
</tr>
<tr>
<td>HL3</td>
<td>2</td>
<td>19</td>
<td>70</td>
<td>4</td>
<td>90</td>
<td>24</td>
</tr>
<tr>
<td>HL-AL4</td>
<td>9</td>
<td>12</td>
<td>19</td>
<td>11</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>HL-CTS</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>15</td>
<td>104</td>
<td>28</td>
</tr>
</tbody>
</table>

1. Results obtained from 479 serum samples tested by the HL test.
2. Number of HL serum samples showing agreement in other two tests.
3. Number of HL serum samples not showing agreement in other tests.
4. Number of serum samples showing agreement with the AL test.
5. Number of serum samples showing agreement with the CT test.

SUMMARY

A preliminary study was made of the hemolytic (HL) test as compared with the agglutination-lysis (AL) and the capillary tube (CT) tests for the serological diagnosis of bovine leptospirosis. The results obtained were based upon sera collected at periodic intervals from experimentally infected animals, and upon serum samples collected from field cases and sent to our laboratory for serological diagnosis.

Using leptospiruria as an indication of active infection, the following results were obtained: (a) The AL test became positive within two weeks post exposure and remained at a diagnostic level long after the animal had recovered from the active infection. (b) Four weeks post exposure were required for the CT test to become positive in a few animals and it became negative as early as eight weeks post exposure, but the titer remained at a diagnostic level for at least 20 weeks in 25 percent of the animals. (c) Not all animals developed a positive reaction to the HL test; those animals that developed leptospiruria showed a positive HL reaction. The HL test became negative within eight weeks after cessation of demonstrable leptospiruria.

The results obtained from individual serum samples sent to our laboratory for serological diagnosis were as follows: (a) If they were positive to the HL test, they were also positive to the CT and AL tests. (b) If they were positive to the CT they would also be positive to the AL, but not necessarily to the HL. (c) If they were negative to the AL they would also be negative to the other two. (d) If they were negative to the CT they would also be negative to the HL, but not necessarily to the AL.

REFERENCES

NATIONAL SURVEY OF SEROLOGICAL TECHNICS USED FOR
THE DIAGNOSIS OF LEPTOSPIROSIS

E. A. CARBREY, V.M.D., M.S., Ames, Iowa

Any efforts directed toward the development of a standard method for
the diagnosis of leptospirosis must begin with an investigation of the ser-
ological procedures currently employed. On the one hand, if it is assumed
that all laboratories are performing with 100 percent efficiency, such a
survey would show the natural experimental error of the technics utilized. If,
however, the laboratories are allowing innovations and inaccuracies to in-
fluence their interpretation of serological titers against leptospirosis, the
survey would reveal the offenders and furnish a base line for future evalua-
tion of any efforts directed toward standardization.

With the above objectives in mind, a set of 25 sera was sent to all lab-
oratories willing to participate in the study. A questionnaire accompanied
the test samples so that the variation among laboratories could be studied
in relation to minor technical details. The titers of the sera ranged from
high to low and included one negative serum. Two of the sera were collected
from sheep, four from swine, and the remainder from cattle. Sera from both
naturally and experimentally infected animals were included in the survey.
Definite exposure to L. pomona or actual isolation of L. pneumophila occurred in
fifteen of the animals from which sera were collected. The sera were filter
sterilized before packaging for distribution.

Seventy-three laboratories tested the sera with some performing more than
one type of test (4). In addition to the well-known technics such as the
agglutination-lysis, Stoenner plate (8), Stoenner capillary tube (9), Galton
plate (2), microscopic agglutination with formalin-treated antigen, and com-
plement-fixation tests, there were other technics reported by individual lab-
oratories. These were a test developed by Roth (6), the Howarth tube test
(3), a test utilizing latex particles sensitized with leptospiral antigens de-
developed by Muraschi (5), and the hemolytic test developed by Cox (1). The
results of these tests were adequately reported previously and were not in-
cluded in the statistical treatment of the data because only one laboratory
was involved with each test. One of the two laboratories which reported
the complement-fixation test did not determine end titers, so this technic was
not subjected to statistical analysis.

Analysis of variance was the statistical method employed for the basic
evaluation of the reports from the laboratories. This method permitted the
separation of the total variance into three components. The largest com-
ponent of variation was contributed by the 25 sera which were selected so as
to give a maximum range of end titer values. Another component was that
contributed by systematic variation among laboratories due to differences
in the technic as performed by each laboratory. The remaining component,
which was obtained by subtracting the sera and laboratory variation from the total variation, was designated as the residual variation or experimental error of the serological technic employed. The square root of the residual variance was taken to obtain the standard deviation of the test as it was used by the laboratories on the 25 sera.

The systematic variation among laboratories on the same serological technic was computed by dividing the laboratory component of variance by the residual variance. The value obtained, called the $F$ ratio, was compared with a theoretical one obtained from a statistical table which represented an $F$ value that might be exceeded by chance alone one percent of the time. If the $F$ ratio between laboratory variance and the residual variance exceeds the $F$ ratio taken from the table, it may be concluded there has occurred a highly significant degree of variation in the application of the test by the individual laboratories in the sense that this variation can not be attributed to chance alone.

In order to apply the analysis of variance to the serological data, it was first necessary to transform the dilution values into logarithms (to the base 10) to make them additive. Negative readings were assigned a logarithmic value of 0.30 corresponding to a titer of 1:2, a generally acceptable nonspecific level of antibody. In the tests where a variety of dilution schemes were employed by different laboratories, the logarithm corresponding to the end titer of a serum was increased to a value halfway between the end titer and the next dilution.

In order to gain some idea of the variation existing among repetitions or runs of the technic of one laboratory for comparison with the variation to be measured among laboratories, five complete runs were made on the 25 test sera using the Stoenner plate test. The analysis of variance is summarized in Table 1 and furnishes a good example of the method used. The first column from the left lists the sources of the variation as described previously. From the next column it is obvious that the degrees of freedom are simply one less than the number of runs or sera. The values in the third column, Sum of Squares, are divided by the appropriate degrees of freedom to produce the mean squares which are recorded in the fourth column. These mean square values are used in computing the $F$ ratio. The mean square for Runs divided by the Residual mean square resulted in an $F$ value of 3.22, which was less than the theoretical $F$ of 3.51 at the one percent level (7). However, the proximity of the calculated $F$ to the one percent level indicated a high degree of systematic variation among runs in this little experiment. Variation which could have occurred by chance alone one time out of one hundred indicated highly significant differences among runs. Compared with other biological technics that have been studied with analysis of variance, this degree of variation was not unusual and gave us a convenient yardstick for comparison with the $F$ ratios calculated for laboratory variation in our survey.

To facilitate the analysis of variance on the large mass of data consisting of 35 Stoenner plate tests, 28 agglutination-lysis tests, eight capillary tube tests, six Galton tests, and three microscopic agglutination tests all on 25
sera; the logarithms of the end titer values were punched on IBM cards and the analysis of variance calculated by a 650 Computer. The F, or variance ratios, and the standard deviations of the five serological technics are shown in Table 2. Compared with the variance ratio obtained for runs in Table 1, the F ratios for laboratory variation were extremely large. Of the five serological tests, the poorest showing was made by the Microscopic Agglutination test (formalin-treated antigen) where the laboratory variation was greater than the Sera variation. Considering the theoretical F values at the one percent level placed in the lower right corner of each block, the laboratory variation of the agglutination-lysis test, 28.41, was greater than that of the Stoenner Plate test, 20.18 (7). The best showing was made by the laboratories using the Galton technic which showed an F of 8.70 against a one percent level of 3.17 indicating the lowest degree of systematic variation among laboratories. This lower systematic variation may have reflected many things, such as better initial training of personnel or a serological technic that was more reproducible. An obvious facet of laboratory variation was the dilution protocols used. The Galton test was the only one of the five which described a standard dilution protocol and actually specified the size of pipet and volumes of diluent. This may have contributed to lower variation among laboratories using this technic. The sera variance ratios were quite large since this was a basic part of the design of the survey. The most revealing part of the analysis concerned the standard deviations of the serological technics. The standard deviation of the agglutination-lysis was about a five-fold dilution, while the rest of the technics using killed leptospiral antigens had a standard deviation of about a two-fold dilution. Since

![Figure 3: Galton Plate Test Laboratory Means]

Grand Mean 1.66

<table>
<thead>
<tr>
<th>Laboratory Code Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>65 56 59 57 61 75</td>
</tr>
</tbody>
</table>

Each bar connects laboratory means not significantly different from one another at 5% level.
there was little difference among the four tests using killed antigens as to standard deviation which, in itself, was actually a measure of the experimental error; it was concluded that in this survey the tests were of equal worth and no technic could be singled out as inherently more accurate than the others. It should not be concluded from this table that the Galton test was the best technic. Rather, it should be said that the laboratories using the Galton test differed least in their test procedures.

A further analysis of the data was performed to determine which laboratories contributed the most variation and which groups of laboratories were not significantly different from one another. In Figure 3 the laboratory means (each laboratory mean was the sum of the logarithms of the end titers of the test sera divided by 25) for the Galton test are arranged on a linear scale. The mean value and the code number of the laboratory are indicated and the laboratories whose mean values are not significantly different at the five percent level are joined by the bars seen in the lower part of the figure. This is an application of the Q test for significant differences among group means that have shown a high F ratio (7). The distribution was slightly unbalanced with the largest group of laboratories not significantly different from one another located above the grand mean. Laboratory 65 was consistently low on all sera and recorded a high titered serum as negative.

The laboratory means for the Stoenner Capillary Tube Test are displayed in Figure 4. In this test the largest group of laboratories that were not significantly different, laboratories 80, 29, 43, and 44, were located above the grand mean. The means of both laboratory 36 and 51 were significantly different from the rest of the laboratory means. Laboratory 51 employed a different dilution scheme. Laboratory 36 determined end titers by reading 100 percent agglutination instead of the correct method in which the end
titer would be the highest dilution showing at least 1+ agglutination. However, to confuse the issue, laboratories 37 and 43 also read 100 percent agglutination as the end point, but came closer to the grand mean.

Figure 5 presents a similar view of the Stoenner Plate Test. Except for a few laboratories, the means were fairly well grouped together with the largest group of laboratories not possessing significant differences located below the grand mean. Laboratory 46, whose mean was considerably out of range, prepared its own antigen for the test. This antigen gave 1-160 titer with the negative serum, No. 10. Two other laboratories, 45 and 49, whose means were in the high range, prepared their own antigen. Laboratory 79 used magnification to read the test, thereby pulling its mean into a higher value. The low mean of laboratory 38 may have been due to some clerical error, since the laboratory reported sera No. 6 through No. 12 as negative. The end titers of the rest of the sera looked quite reasonable. Laboratories 20 and 30, whose means were on the low side of the scale, used different dilution schemes than the one recommended by Stoenner. Laboratory 42 was of interest, in that it reported the use of an antigen lot that was two months outdated. The mean of this laboratory, 1.85, was very close to the grand mean, 1.83.

The next question which was presented was how much of the variation among the laboratory means was due to differences in the antigen. If there were variations in the sensitivity among different lots of antigen or among antigens made by different producers, this would have increased the variation among laboratories. To check this point, the means of the laboratories employing the Stoenner Plate test were subjected to further analysis using a hierarchal classification or nested sample technic (7). This method of analysis permitted the separation of the variance among laboratory means into two separate components; one contributed by variation between producers.
and the other by variation among lots of antigen (antigen lot as identified by serial number). There were two producers, one represented by eleven lots and the other by three lots. The laboratories which made their own antigen were excluded from this analysis. Tables 5.1 and 5.2 summarize this analysis of variance. The mean square for Producers divided by the mean square for Lots in Producers yielded an F ratio which represented the systematic variation between producers. An F value of 0.56 indicated that no significant component of variance was contributed by the producers. The ratio of the mean square for Lots in Producers to Laboratories in Lots represented the component of variance contributed by differences among serial number lots of antigen. An F of 1.87 was calculated and, compared with the theoretical F of 2.34 at the five percent level, was not considered significant. From the magnitude of this F value, 1.87, it may be possible that definite differences in sensitivity occurred among the lots of antigen. However, the laboratory variation was so large that the contribution made by the different antigen lots was small by comparison.

It was felt after a review of the questionnaires that the laboratory variation on the Stoenner Plate test was related to the lack of a standard dilution protocol for achieving the dilution titers recommended by Stoenner. Although other aspects of the technic were uniform; it would probably help to have each laboratory visited periodically by some competent person to detect hidden sources of variation which were not revealed by the questionnaire.

![FIGURE 6](image.png)

**Agglutination-lysis Test**

**Laboratory Means**

Grand Mean 3.54

Aside from the main theme, it was of interest to investigate the applications made by the laboratories of the Stoenner Plate and Capillary Tube
E. A. CARBREY

tests. This material is summarized in Table 5.3. Out of a total of 42 laboratories, 11 stated that they did not report end titers to the practising veterinarian or physician.

The agglutination-lysis test provided a fascinating problem in analysis with the many factors contributing to laboratory variation. Figure 6 shows the distribution of laboratory means on a linear scale with bars connecting those means which were not significantly different. Unlike the other serological tests the largest group of laboratories, fifteen, were located symmetrically about the grand mean. Laboratory 53, at the lower end of the scale, employed a strain of *L. pomona* for antigen which had been isolated at that laboratory. It was not stated in the questionnaire whether this strain, named Schlitz, had been checked out by one of the leptospirosis reference laboratories. Laboratory 50 at the extreme upper end of the scale used a greater than ten-fold dilution scheme which involved the use of a 0.2 ml pipet transferring 0.01 ml aliquots. The rinsing of serum proteins from this small pipet and the carrying of significant amounts of inoculum on the external surface of the pipet may have contributed to the high mean of this laboratory.

The questionnaires submitted by the laboratories with the test results were used to gain some indication as to which variables in the agglutination-lysis test caused significant differences among laboratories. The standardization of the live antigen used in the test was accomplished by darkfield examination in 26 out of 28 laboratories. Luxuriant cultures were diluted to obtain an antigen of suitable density as observed by either high or low power. One laboratory reported standardization of the antigen by light transmission and the other laboratory used a Petroff-Hauser counting chamber to count the leptospira in their antigen. About half of the laboratories reported the use of centrifugation to "clear" the antigen. The greatest variations occurred in regard to dilution scheme, antigen strain, incubation temperature of the test, and culture medium. A comparison among the means was made in regard to these variables.

Table 6.1 presents a comparison among laboratory means of laboratories using the same dilution scheme. The mean values in the table were obtained by dividing the sum of the means of all the laboratories using the same dilution scheme by the number of laboratories. It was observed that the ten-fold dilution scheme mean was higher than that of the other two dilution schemes. This difference was further developed in Table 6.2 by making a direct comparison between ten-fold and the other dilution schemes. The difference between the two means, 0.643, was divided by the sample standard error to compute a t value of 2.907. This number was compared with t values from a statistical table to determine the probability of obtaining a t of this magnitude by chance alone. The probability of obtaining a t value of 2.907 was one percent indicating a highly significant difference between the ten-fold dilution scheme and the others. This was considered a reasonable conclusion in that a pipet would be rinsed more frequently in preparing two or four-fold dilutions than in preparing 10-fold dilutions to obtain a given end titer. All but one laboratory reported the use of the same pipet in
preparing dilutions on a serum sample. The use of separate pipets for each dilution would be an expensive alternative.

The strain of *L. pomona* used in the preparation of the antigen was found to contribute a component of the variation among laboratories. The most popular strain was the Johnson strain. Table 6.3 shows the comparison among means of laboratories using the same antigen strain. The mean of the Johnson strain was high, 3.958, while the means of the Pomona, S-91 and the Miscellaneous groups were lower and lay closer together. In Table 6.4 the comparison between the Johnson strain and the others as a group is presented. The difference between the two means, 0.523, produced a t value which was significant at a probability level of 2.2 percent. This indicated a significant contribution to the variation among laboratory means by the Johnson strain.

Table 6.5 shows the comparison among means grouped according to the incubation temperature of the test. A mean of 4.013 was computed for laboratories using an incubation temperature of 37°C. This mean was somewhat higher than the group means for Room Temperature and 28-32°C. A further comparison is presented in Table 6.6. The difference between the group means for an incubation temperature of 37°C as compared to other incubation temperatures was found to be 0.509. A t value was computed from this difference which was significant at a probability level of 4.1 percent.

The comparison of laboratory means by the culture medium used in the preparation of the antigen is presented in Table 6.7. There was no significant contribution by culture medium to the variation among laboratory means. Ideally a study of these four factors as sources of variation should have been carried out in one large analysis comprising all four factors, but with the present incomplete data such an analysis would have been extremely involved.

Analysis of the data has shown that definite contributions to the variation among laboratory findings on the test sera were made by the use of different dilution schemes, antigen strains and incubation temperatures. Standardization of the agglutination-lysis test must depend on the adoption of uniform procedures in regard to these three attributes.

One of the problems of leptospirosis diagnosis today is how the serum titers reported on the different serological tests should be interpreted. The desire to create an analogy with the "reactor" titer of the brucellosis test is both natural and tempting. A "reactor" titer for leptospirosis diagnosis would furnish a necessary constant for the establishment of regulations. Unfortunately, the relation of a particular antibody titer to the infective stage of the disease has not been decided to the satisfaction of everyone. From the data accumulated in this survey, we can approach this problem from a different angle. The standard deviation of a serological technic represented its inherent experimental error as it was applied by all of the laboratories. Now, if a titer were selected which was generally considered a nonspecific level of antibody, the standard deviation may be used to calculate a minimum titer value which would be based on the experimental error of the technic. If the minimum titer were selected so that it was exactly two standard deviations
above the nonspecific titer level, then a 95 percent confidence interval would have been created. When this minimum titer is observed as the reacting titer there would still be a one in forty chance of incorrectly designating an animal as infected when the antibody content of the serum is actually at the nonspecific level. The minimum titers for four of the tests have been calculated and are shown in Table 7.0. Of course, these minimum titers have no relation to the biological picture, but are based simply on the experimental error of the serological technics. The nonspecific titer levels were selected arbitrarily and are open to criticism. Using the standard deviation for the agglutination-lysis test of a 5.14 dilution factor, selection of a nonspecific titer level of a 1-100 dilution produced a minimum titer at the 1-1028 dilution. An alternative minimum titer of 1-257 was computed for the lower nonspecific level of 1-25. The minimum titers for the two Stoenner tests were calculated to be 1-48 and 1-56 which were quite close to the standard 1-40 dilution used in these technics. The diagram in Figure 7.1 shows the relation of the two titer levels in the Stoenner Plate test. Titers in the range between the nonspecific titer and the minimum titer may be classified as suspicious.

The proposition of the minimum titer as outlined above may be somewhat removed from the natural aspects of the disease and can only serve as an expression of the limitations of our serological technics for the diagnosis of leptospirosis. However, it seems clearly indicated that the reporting of serological titers lower than these minimal titers as positive indications of the disease serves to create false impressions. The so-called reacting titer may be placed anywhere above this minimum level, but certainly cannot be placed below it without implying an accuracy which does not exist in the serological tests employed.

**Summary**

1. A survey of the serological technics used for the diagnosis of leptospirosis was made by sending sets of 25 sera to 73 laboratories. A questionnaire was completed by each laboratory giving the details of the technic or technics used. An analysis of variance performed on the data revealed a high degree of systematic variation among laboratories on the five technics most widely used:

   - Agglutination-lysis
   - Stoenner Plate
   - Stoenner Capillary Tube
   - Galton Plate
   - Microscopic agglutination with formalin-treated antigen

   The lowest degree of systematic laboratory variation was shown by the laboratories performing the Galton test.

   The standard deviation calculated for the agglutination-lysis test was about a five-fold dilution factor. The standard deviations of the other tests were roughly the same, being slightly more than a two-fold dilution factor.
The distribution of the laboratory means of each test were studied and the groups of laboratories whose means were not significantly different from one another were identified.

Analysis of variance on the laboratory means of the Stoenner Plate test was performed and the components of variation contributed by the different producers and different serial lots of antigen were calculated. Neither of these sources introduced significant variation.

Statistical study of the results of the survey of the agglutination-lysis test revealed that the use of different dilution schemes, antigen strains, and incubation temperatures produced significant components of the variation among laboratories.

A minimum titer was computed for each of the serological tests above which a high degree of confidence could be expressed in a positive interpretation of the test. This minimum titer level was located two standard deviations above the nonspecific titer level.

**Acknowledgment**

The author thanks Dr. R. A. Packer and Dr. H. O. Hartley of the staff of Iowa State University for advice and encouragement and Mrs. Helen Sailsbury of the ADE staff for excellent technical assistance.

**TABLE 1**

*Laboratory 80, Stoenner Plate Test*

*Analysis of Variance*

*Twenty-five Sera on Five Runs*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera</td>
<td>25-1 = 24</td>
<td>116.3421</td>
<td>4.848</td>
</tr>
<tr>
<td>Runs</td>
<td>5-1 = 4</td>
<td>0.1776</td>
<td>0.0444</td>
</tr>
<tr>
<td>Residual (Error)</td>
<td>(4) (24) = 96</td>
<td>1.3238</td>
<td>0.0138</td>
</tr>
<tr>
<td>Total</td>
<td>125-1 = 124</td>
<td>117.8435</td>
<td></td>
</tr>
</tbody>
</table>

\[ \frac{0.0444}{0.0138} = 3.22 \text{ as compared with } F = 3.51 \text{ at the 1 percent level} \]

(indicating one chance in a hundred in this experiment of exceeding an F value of 3.51)
### TABLE 2

<table>
<thead>
<tr>
<th>Test</th>
<th>Variance Ratio Among Laboratories</th>
<th>Variance Ratio Among Sera</th>
<th>Standard Deviation Expressed as a Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 Agglutination-Lysis</td>
<td>28.41 *1.81</td>
<td>72.44 *1.83</td>
<td>1—5.14</td>
</tr>
<tr>
<td>35 Stoenner Plate</td>
<td>20.18 1.71</td>
<td>181.02 1.82</td>
<td>1—2.40</td>
</tr>
<tr>
<td>8 Stoenner Capillary Tube</td>
<td>30.96 2.75</td>
<td>41.25 1.90</td>
<td>1—2.81</td>
</tr>
<tr>
<td>6 Galton Plate</td>
<td>8.70 3.17</td>
<td>19.82 1.92</td>
<td>1—2.53</td>
</tr>
<tr>
<td>3 Microscopic Agglutination</td>
<td>46.66 5.08</td>
<td>26.07 2.20</td>
<td>1—2.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* F value which one would expect to exceed by chance alone 1 percent of the time.

### TABLE 5.1

**Stoenner Plate Test**

**Analysis of Variance, Laboratory Means**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Producers</td>
<td>1</td>
<td>0.0472</td>
<td>0.04720</td>
</tr>
<tr>
<td>Lots in Producers</td>
<td>12</td>
<td>1.0074</td>
<td>0.08395</td>
</tr>
<tr>
<td>Laboratories in Lots</td>
<td>18</td>
<td>0.8061</td>
<td>0.04478</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>1.8607</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 5.2

**Stoenner Plate Test**

**Systematic Variation Between Antigen Producers**

\[
F = \frac{0.04720}{0.08395} = 0.56 \text{ as compared with } F = 4.75 \text{ at 5 percent Level}
\]

\[
F = \frac{9.33}{1} = 9.33 \text{ at 1 percent Level}
\]

**Systematic Variation Among Antigen Lots on the Same Producer**

\[
F = \frac{0.08395}{0.04478} = 1.87 \text{ as compared with } F = 2.34 \text{ at 5 percent Level}
\]

\[
F = \frac{3.37}{1} = 3.37 \text{ at 1 percent Level}
\]
TABLE 5.3

Stoenner Plate and Capillary Tube Tests

| Laboratories determining titers on all sera reacting to the screen test | 35 |
| Laboratories not determining titers | 7 |
| **Total** | **42** |

Thirty-one out of the 35 laboratories performing end titer determinations report the results to the veterinarian or physician.

TABLE 6.1

Agglutination-Lysis Test

Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Dilution Scheme</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 fold</td>
<td>3</td>
<td>3.333</td>
</tr>
<tr>
<td>4 fold</td>
<td>8</td>
<td>3.264</td>
</tr>
<tr>
<td>10 fold</td>
<td>13</td>
<td>3.926</td>
</tr>
</tbody>
</table>

TABLE 6.2

Agglutination-Lysis Test

Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Dilution Scheme</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 fold</td>
<td>13</td>
<td>3.926</td>
</tr>
<tr>
<td>2 fold and 4 fold</td>
<td>11</td>
<td>3.283</td>
</tr>
</tbody>
</table>

\[ t = \frac{0.643}{S_x} = 2.907 \quad \text{Probability} = 1\% \]

\( S_x \), or sample standard error, computed from a factorial comparison of Dilution Scheme versus Incubation Temperature.

TABLE 6.3

Agglutination-Lysis Test

Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Antigen Strain</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson</td>
<td>9</td>
<td>3.958</td>
</tr>
<tr>
<td>Pomona</td>
<td>5</td>
<td>3.396</td>
</tr>
<tr>
<td>S-91</td>
<td>4</td>
<td>3.472</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>6</td>
<td>3.443</td>
</tr>
</tbody>
</table>
TABLE 6.4
Agglutination-Lysis Test
Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Antigen Strain</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson</td>
<td>9</td>
<td>3.958</td>
</tr>
<tr>
<td>Other Strains</td>
<td>15</td>
<td>3.435</td>
</tr>
</tbody>
</table>

\[ t = \frac{0.523}{S_x} = 2.632 \quad \text{Probability} = 2.2 \text{ percent} \]

\( S_x \) or sample standard error, computed from a factorial comparison of Antigen Strain versus Incubation Temperature

TABLE 6.5
Agglutination-Lysis Test
Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Incubation Temperature</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature</td>
<td>11</td>
<td>3.569</td>
</tr>
<tr>
<td>28°-32°C.</td>
<td>7</td>
<td>3.401</td>
</tr>
<tr>
<td>37°C.</td>
<td>6</td>
<td>4.013</td>
</tr>
</tbody>
</table>

TABLE 6.6
Agglutination-Lysis Test
Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Incubation Temperature</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C.</td>
<td>6</td>
<td>4.013</td>
</tr>
<tr>
<td>Room Temperature and 28-32°C.</td>
<td>18</td>
<td>3.504</td>
</tr>
</tbody>
</table>

\[ t = \frac{0.509}{S_x} = 2.292 \quad \text{Probability} = 4.1 \text{ percent} \]

\( S_x \) or sample standard error, computed from a factorial comparison of Antigen Strain versus Incubation Temperature

TABLE 6.7
Agglutination-Lysis Test
Comparison Among Laboratory Means

<table>
<thead>
<tr>
<th>Medium</th>
<th>Number of Laboratories</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuarts</td>
<td>11</td>
<td>3.504</td>
</tr>
<tr>
<td>Schuffners</td>
<td>8</td>
<td>3.640</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5</td>
<td>3.896</td>
</tr>
</tbody>
</table>
TABLE 7.0
Minimum Titers at 95 percent Confidence Level
Based on Standard Deviation of Test

<table>
<thead>
<tr>
<th>Test</th>
<th>Minimum Titer</th>
<th>Standard Deviation (Twice Standard Deviation of Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonspecific Titer</td>
<td></td>
</tr>
<tr>
<td>Agglutination Lysis</td>
<td>5.14</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25)</td>
</tr>
<tr>
<td>Stoenner Plate</td>
<td>2.40</td>
<td>10</td>
</tr>
<tr>
<td>Stoenner Capillary Tube</td>
<td>2.81</td>
<td>10</td>
</tr>
<tr>
<td>Galton Plate</td>
<td>2.53</td>
<td>11.9</td>
</tr>
</tbody>
</table>

TABLE 7.1
Stoenner Plate Range

<table>
<thead>
<tr>
<th>Nonspecific Level</th>
<th>Minimum Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>48</td>
</tr>
</tbody>
</table>

Nonspecific Titers: 
- Suspicious Titers: (considering only experimental error of serological technic)

s = standard deviation

REFERENCES
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

E. E. Roth, Chairman, Baton Rouge, Louisiana; E. H. Bohl, Columbus, Ohio; H. S. Bryan, Kalamazoo, Michigan; E. A. Carbrey, Ames, Iowa; M. J. Cerosaletti, Albany, New York; J. A. Howarth, Davis, California; D. E. Hughes, Beltsville, Maryland; E. V. Morse, Ames, Iowa; J. F. Quinn, Lansing, Michigan; W. L. Sippel, Kissimmee, Florida; C. J. York, Indianapolis, Indiana.

The initial report of this Committee summarized available information and indicated areas where additional study was needed. Last year's report dealt with problems in diagnosis, prophylaxis and control. This report deals with the problems of current special interest and new observations of sufficient importance to merit inclusion.

A number of tools are available which may be used as an aid in the diagnosis of leptospirosis. The serologic procedures fail to differentiate past from present infection. Furthermore, the presence of serum agglutinins has not been shown to be correlated with the carrier state. Recent work with the Hemolytic test suggests a significant degree of correlation with shedding of the organisms in the urine to warrant further consideration. In addition, the application of the fluorescent antibody technique for the detection of leptospires in tissues and urine has been reported. Further development and refinement of these methods may prove them to be valuable for the diagnosis of infection.

The study reported by Carbrey emphasizes once again the lack of uniformity of serological diagnostic procedures in current use. The following recommendations are made with the hope that a satisfactory degree of uniformity can be achieved through the use of standard technique.

1. Stoenner Test.

It is essential to follow the protocol that Stoenner described in the Am. J. Vet. Res., Vol 15, 434-439, 1954. The dilution scheme is considered to be a source of variation; therefore the following dilution scheme is recommended:

(1) Use a one ml. serological (T.D.) pipette with 0.01 graduations.
(2) Place 0.8 ml. saline in the first dilution tube and 0.6 ml. in the subsequent dilution tubes.
(3) Place 0.2 ml. serum in the first tube; mix thoroughly; take up 0.23 ml., place 0.03 ml. on the plate and transfer the remaining 0.2 ml. to the second tube; mix thoroughly; and repeat as above through the remaining dilution tubes. The serum dilutions are 1:5, 1:20, 1:80, and 1:320 which after addition of 0.03 ml. antigen results in dilution of 1:10, 1:40, 1:160 and 1:640.
2. Galton Plate Test.

The protocol is adequately described and should be followed in detail as outlined in the brochure accompanying the antigens.

3. Agglutination-lysis Test.

These recommendations are intended to serve as a baseline for the performance of the agglutination-lysis test.

a. The following system of serum dilution should be used:
   (1) Prepare serum dilutions of 1:50, 1:500, 1:5000, and 1:50,000. After mixing an equal amount of each serum dilution with antigen, the final dilutions are 1:100, 1:1000, 1:10,000 and 1:100,000.
   (2) Dilutions should be prepared using Screnson's phosphate buffer at PH 7.4 containing 0.85 percent sodium chloride; and
   (3) Use a 1 ml. serological pipette.

b. The test should be incubated for two hours at 37 C.

c. The reaction should be estimated as the degree of clearing when compared to the antigen control and a 50 percent reaction should be considered as positive for the particular dilution.

d. Antigen used for the test should be:
   (1) The type strain L. pomona (Pomona).
   (2) Grown in Stuart's medium enriched with 7-10 percent rabbit serum.
   (3) Three to five day old cultures started by inoculating with 10 percent of an actively growing culture incubated at 28-30 C. Cell counts as determined using a Petroff-Hauser counting chamber should fall within a range of 175 to 225 million organisms per milliliter.
   (4) When the culture requires dilution use uninoculated Stuart's medium.

e. A darkfield microscope system providing a magnification of approximately 150X should be used.

f. Both negative and positive serum controls should be used routinely at the same dilutions as the test.

The position regarding the suggested use of L. pomona bacterin in cattle and swine has not changed since last year's report. However, it should be noted that reports of anaphylactoid reactions in revaccinated cattle have been received. These reactions have been seen in a small percentage of the total number of cattle revaccinated; however, within certain herds, it may involve a large percentage. The severity of the reactions vary from a mild reaction with slight trembling accompanied by edema of the vulva and periocular tissues to a severe reaction with acute respiratory distress. This may be attributed to the high level of rabbit serum in the bacterin or to the antigenicity of the organism itself.
Animals other than domestic livestock in which *L. pomona* has been found to occur naturally include dog, deer, skunk, raccoon, bobcat and opossum. Preliminary investigations in Georgia and Louisiana indicate that the incidence of *L. pomona* infection in skunks is significantly higher than in other animals mentioned. These foci of infection may explain the source of new infections in cattle and swine where no other opportunity of infection is known.

*L. pomona* is presently considered the primary cause of leptospirosis in cattle and swine.

Recent isolation of *L. hardjo* from one herd of clinically normal cattle in Louisiana and *L. canicola* from one herd of cattle in Alabama exhibiting signs of disease emphasizes that other serotypes may be involved in bovine leptospirosis.

Reactions to still other serotypes are being found but at the moment their significance remains unexplained. The cause and exact nature of these reactions should be critically investigated. At the present time vaccination with these serotypes does not appear justified.

It is premature to consider a regulatory control program for leptospirosis on a national, area, or state basis. No practical method can be recommended that will detect the carrier animal. The presence of *L. pomona* infection in wild animals emphasizes that the disease is not limited to domestic animals, hence it cannot be controlled by quarantine. The recent reports of the occurrence of other serotypes indicate that control programs based on *L. pomona* would be inadequate.

It can be noted from this report that research efforts are being directed to many areas where information is now deficient. No doubt our knowledge will increase as a result of existing as well as future investigations. We recommend that the work of this Committee be continued.
Since both dairy cattle and beef cattle are of primary importance in providing food for human beings, all persons concerned with the production of beef and dairy products should have a vital interest in promoting greater productivity of cattle, and any information that helps should be welcomed. Research programs on feeding, breeding, or cattle husbandry that tend to help productivity are supported vigorously, and because their application is expected to return greater profits, new suggestions are received enthusiastically. New methods can be quickly evaluated merely by observing the increased yield of each animal.

Although veterinary science is an important part of this effort, workers in this field apparently have not established the same close relationship with cattle producers. In the process of disease control, economic benefits result from preventing losses. The end result to the cattle producer of increased yield is just as important as that produced by better feeding methods. But, unfortunately, the producer himself cannot observe the increased yield of each individual animal from disease prevention and, as a result, veterinary information does not seem to be regarded as economically beneficial by producers.

Veterinary research can present a program that offers possibilities of being economically beneficial to the producer of cattle. As a result of application of new techniques, the causative agents of a number of diseases have been revealed recently. With these new techniques, vaccines which will prevent these diseases have been made and tested in the research laboratory. But will these vaccines be made, and will they give satisfaction in use? Only if cattle producers themselves demand their use extensively, and they are proven to be of economic benefit.

Vaccination must not be presented primarily as a program to prevent diseases. As such, the choice of whether to vaccinate or not is left to the cattle producer. The cattle producer is not trained in veterinary science and, lacking this background, often decides not to vaccinate, thinking that by escaping a particular disease during a particular year, profits are greater because the cost of vaccination has been saved. Sometimes, this appears to be true unless annual carryover of cost of vaccination is balanced against long-range losses from disease. To overcome this false thinking, evidence of an economic nature must be produced, showing that a vaccination program, correctly instituted and precisely followed, increases profits. The economic
value of vaccination, therefore, is conceived as a positive approach for the cattle producer to consider rather than the negative one of disease prevention.

This paper is intended to suggest a vaccination program and method for economic evaluation, with the hope that the time is here—now—for a veterinary program to go forward positively and achieve enthusiastic support of cattle producers.

**NEWER KNOWLEDGE ABOUT CATTLE DISEASES**

The development of knowledge about infectious diseases has followed a most interesting pattern. First, of course, the infectious nature of disease was recognized. Later, microbial causation was suggested, but to prove this, new techniques were required for bacterial cultivation. Experimental methods had to be formulated to show that the bacteria which had been cultivated really caused the disease under study (Koch’s postulates). Application of bacteriological methods, however, failed to account for all diseases, until eventually, viruses were discovered. Again, new techniques became necessary. Obviously, progress has resulted from application of techniques. In the virus field, progress came with transfer of virus to laboratory animals, then by cultivation in eggs, and, finally, tissue culture to furnish viruses for purification, study by chemical methods, and for vaccination.

In the veterinary sense, changing concepts of disease paralleled increasing knowledge of causative agents. Initially, pathological conditions were recognized by observation, and diagnoses such as pneumonia, diarrhea, etc., were given. After discovery of agents causing disease, it was recognized that in different individuals a particular pathogen could cause variations of effects from mild to severe. In addition, further knowledge revealed that a particular condition might result from one of several possible causes, such as brucellosis abortion, leptospiral abortion, etc. Now, we must also consider that one particular pathogen can cause a variety of different pathological conditions. This is best illustrated by the IBR-IPV virus, which can produce such diverse effects as pneumonia and vaginitis.

The structure of the body is simple in the sense that there are only a few functional systems. Since there are a larger number of pathogens than systems, with the capacity of these pathogens to affect several systems, clinical diagnosis for specific therapy becomes difficult without laboratory aid. Unfortunately, laboratory diagnosis for an individual case usually is retrospective and, thus, becomes of educational value only. It, therefore, becomes imperative that the problem of infectious disease control be considered in the larger sense of prophylaxis instead of treatment. Laboratory aid then becomes of real value, in that incidence studies define disease problems, and vaccination becomes a tool for disease prevention.

For this presentation about vaccination, present knowledge is reviewed on the basis of etiology rather than pathology. Furthermore, except for serological tests, only information considered pertinent to vaccination is presented. Additional details may be procured by consulting the references cited in the bibliography.
Vaccination of Cattle for Increased Profits

Leptospira pomona. After the isolation of a leptospira from cattle and its identification as *L. pomona* (1), it was not long before incidence studies suggested the need for control measures. Not only was the incidence found significant by serological tests, but losses in milk production, abortion, growth retardation, and some mortality were all convincing proof that something must be done to reduce the effects being produced by this disease.

Both chemotherapy and vaccination were evaluated, and, thus far, principal reliance for control has been placed on a bacterin. While attenuated viable organisms would seem better for producing more durable immunity, these particular organisms do not survive drying from the frozen state, hence only a bacterin has been developed for this particular disease. Considerable study has indicated its value as a preventative.

Virus diarrhea. First reported in 1946 (2, 3) and later included for clinical discussion with similar clinical and pathological conditions which became known as mucosal disease complex (4), this virus disease has been shown to have a widespread distribution in the United States (5, 6, 7, 8). Unfortunately, nomenclature and, consequently, diagnosis have been based on pathological findings of certain characteristic ulcerative lesions. Thus, the impression gained by clinicians was that of a disease with sporadic occurrence.

Laboratory-produced disease did not resemble that reported as characteristic, yet these findings carried the implication that this disease may have a higher incidence than was recognized. Lacking a serological test, there could be only confusion and, even though a suitable vaccine virus for prevention has been available since 1954 (5), no effort to vaccinate has been made in spite of the early suggestion that abortion also may be a manifestation of virus diarrhea.

Efforts at tissue culture were successful but no cytopathogenic effects were noted with the first two strains tested (9, 10). Then, recently, a strain obtained from a calf in Oregon and four additional strains from New York cattle produced cytopathogenic effects and a serological and immunological test was demonstrated (11). A preliminary study for incidence in New York cattle already has shown a rate in excess of 50 percent in dairy cows, and this virus has been implicated as the cause of abortions in two herds (12). It is interesting that information necessary for successful control has been produced by research even before vaccination has been suggested.

Infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV) virus. Initial isolation of this virus by tissue culture methods came from cattle that showed a rhinotracheitis under western feedlot conditions (13). Fortunately, IBR-IPV virus could be neutralized by serums from immune cattle, thus providing a serological test for diagnosis as well as a test for immunity. Using this test, infection with this virus was shown to occur in about five percent of eastern cattle, yet rhinotracheitis was not recognized (14). Then, an infectious pustular vulvovaginitis was defined (15) and shown to be caused by the same virus (16), but incidence of this clinical manifestation also was insufficient to account for the amount of disease indicated serologically.
Then, virus was fed newborn calves and caused illness and death with lesions in the mouth, esophagus and forestomachs (17). Recovery was complicated by pneumonia. Also, virus placed on the conjunctiva produced a conjunctivitis (18) and, when inoculated into the teat cistern, mastitis occurred (19). The IBR-IPV virus, therefore, has a predilection for mucosal surfaces, and manifestation of disease coincides with portal of entry of the virus.

Already, IBR-IPV virus has been shown to be widespread, and sufficiently important to be prevented on the basis of recognized naturally occurring disease. Laboratory produced disease indicates only partial knowledge and this virus should be considered as the cause of any infectious condition of cattle showing mucosal lesions, especially those of newborn calves. Complete knowledge would enhance the value of prevention and since an effective vaccine already is available (20), control could proceed rapidly.

*Miyagawanella bovis.* The first indication of an elementary body virus infecting calves came with the description of sporadic bovine encephalomyelitis in Iowa (22). Eventually, it was related to the psittacosis-lymphogranuloma (P-LV) group (22). This condition continues to occur sporadically (23). Meanwhile, studies which involved inoculation of fecal suspensions from calves into guinea pigs revealed a new member of the P-LV group as a common infection of the intestinal tract of New York calves (24). No signs of illness were shown when calves that were four to nine months of age were infected, but new born calves from non-immune mothers or calves from immune mothers that were deprived of colostrum showed diarrhea and occasionally died (25). No signs of illness were seen if calves received colostrum from immune mothers. Inoculation of this virus into the teat cistern of lactating cows produced mastitis, but it has not been related to mastitis in cows under barn conditions (26). More recently, in the State of Washington, a pneumonitis has been produced in calves with a virus belonging to the P-LV group (27).

An adequate comparison has not been made of the serological relationship between the enteric, neurological and respiratory strains. Of course, the complement fixation test would reveal no difference since all members of the P-LV group cross react by this test. It would be expected that all strains would be antigenically similar and the variety of clinical manifestations are caused by the same virus. Further study must be done before consideration can be given for a vaccine. Furthermore, with or without recognized pathology, an evaluation of the effects of this virus on growth of calves would be desirable. Obviously, this virus is widespread and conceivably a good vaccine would be of material benefit. Effective vaccines have been made for other members of the P-LV group and this member should be amenable to the same process (22).

*Parainfluenza 3 virus.* Only recently isolated from cattle showing respiratory disease (28, 29, 30), this virus is receiving intensive study as the primary cause of shipping fever. Inoculation of calves produces a mild transient illness. While not implicated conclusively, secondary invader organisms such as *pasteurella* and pleuropneumonia may combine with this virus to produce the clinical syndrome seen in feedlots.
There is a serological test which is reliable for diagnosis (31), although insufficiently studied for immunological purposes. Serial samples tested thus far have indicated a relationship of this virus to respiratory disease seen in feedlots, although it did not account for all (29). Indeed, IBR virus has been shown to be a feedlot respiratory infection (13) and *M. bovis* has been suggested (27).

*A serological service for cattle.* The inability to assess incidence of disease by clinical means requires another method. While there is no perfect method, if their limitations are understood and corrected, serological tests for antibodies will give a good appraisal. Obviously, animals that died would be excluded serologically since there must be survival for antibodies to form. Thus, a disease that has a high mortality would show a lower incidence serologically than another with the same incidence but no mortality. Failure of antibodies to persist, for a particular disease, would give a negative test and would not indicate all of the animals that actually had experienced the disease. For cattle, these limitations are not serious and a correction factor can be used if necessary. A serological survey, therefore, can provide essential information to appraise economic effects of disease. In addition, it is useful for diagnosis and, if the serological test is related to immunity, an invaluable tool for measuring efficacy of vaccines is available.

All five of the agents described above lend themselves to at least one type of serological test which has value for incidence and diagnostic work. For *L. pomona*, IBR-IPV virus and VD virus serological findings have been related to immunity while the other two have not been studied sufficiently as yet. There follow, in outline form, serological methods which can be applied to these agents. These methods are the ones used at Cornell and are suggested only as a basis for formulation into a routine serological service for cattle by diagnostic laboratories. It is expected that some revision will be desirable as practical application proceeds.

**VIRUS DIARRHEA NEUTRALIZATION TEST**

*Materials.* It is necessary to have in stock a virus preparation that has been titrated. Diagnostic laboratories may procure seed virus * and produce stock virus for their own use.

Serums are supplied by veterinarians for diagnostic purposes. They should be collected and handled in the same manner as blood samples collected for brucellosis test.

Bovine kidney cell cultures may be prepared by each laboratory unless procured from another source. At Cornell, cells are prepared as follows:

A nearby slaughterhouse furnishes fetuses which are taken from pregnant cows at the time of slaughter. In the preparation of a batch of tubes, a fetus is brought to the laboratory, its kidneys are removed aseptically and placed in a sterile petri dish. Each kidney is sliced longitudinally, its capsule peeled

*If preparation of stock virus is to be done by an individual laboratory, a virus specimen and procedure will be supplied by a request addressed to Dr. James A. Baker, Veterinary Virus Research Institute, Cornell University, Ithaca, New York.*
off and the medulla is removed with curved scissors and discarded. Then the cortex is washed in phosphate buffer solution (PBS), transferred to petri dishes containing a watch glass and, with fine scissors, is minced into pieces two to three cm in size.

The minced tissue is transferred to a 250 ml fluted Erlenmeyer flask containing a magnet. It is washed with PBS until the supernatant is clear (three to five times). The supernatant is decanted after each washing. After the last washing, 75 ml of trypsin is added (Difco 1:250 in Hanks Solution, 0.25 percent). The magnet stirrer is operated for one-half hour, then the trypsin is discarded. One hundred ml of trypsin is added and the unit placed in the refrigerator. The stirrer is operated at low speed overnight (13-16 hours).

Next morning the suspension is divided into two 250 ml centrifuge bottles and each is diluted with 150 ml of PBS. It is centrifuged for five minutes at 800-1,000 r.p.m. The supernatant is siphoned and is discarded. The cell sediment is resuspended in each bottle with a 10 ml pipette and then is centrifuged at 600-800 r.p.m. for three minutes. This washing procedure is repeated until the supernatant is clear (three to five times). The cell suspension is filtered through four layers of cheesecloth fixed over a funnel into a 250 ml Erlenmeyer flask. The filtered suspension is divided between three 50 ml graduated centrifuge tubes and centrifuged exactly at 600 r.p.m. for three minutes and the cell volume is read. Each ml of packed cells is suspended in 250 ml of Hanks medium containing basic buffered salt solution, 0.5 percent lactalbumin hydrolysate, 10 percent horse or bovine serum, and antibiotics (1,000 units of penicillin, 100 micrograms of units, and 100 micrograms of streptomycin per ml).

With a B-D Cornwall automatic syringe 1 ml of cell suspension is placed into a tube, sealed tightly with a white rubber stopper, and each tube is placed in a stationary rack. The rack is handled carefully when placing it in a 36 C incubator so that the position of the tubes is not disturbed.

Usually a fluid change is required three days later. For this, medium is removed with a sterile Pasteur pipette attached to a water siphon. Then, with an automatic syringe each tube is refurnished with 1 ml of Hanks tissue culture medium. After further incubation of two to four days, complete sheets of healthy cells are visible by microscopic examination (figure 1). At this time fluid is again changed and 2 ml of Hanks medium or some other tissue culture medium is added. The cultures are now ready for use and further fluid change is not necessary if the tubes are used immediately and the neutralization test is read within seven days. For growth of cattle viruses, if Hanks medium or Earle’s medium is used, the serum component must be free of antibodies against the test virus. Parker 199 + 0.5 percent lactalbumin, which contains no serum, can be substituted for maintenance of cells but is not as satisfactory.
PROcedure

1. From titrated stock virus (usually $10^{-4.0}$ to $10^{-5.0}$ per 0.1 ml), calculate the dilution necessary to make 100 TCID$_{50}$ per 0.1 ml. Then prepare dilutions with the same medium used for maintenance of cells.

2. Dilution of serum that have been inactivated at 56°C for 30 min. are prepared in serological tubes as follows:

- undil. serum = undil.
- 0.2 ml of serum plus 0.4 ml of medium = 1:3
- 0.2 ml of 1:3 serum dilution plus 0.4 ml of medium = 1:9
- 0.2 ml of 1:9 serum dilution plus 0.4 ml of medium = 1:27
- 0.2 ml of 1:27 serum dilution plus 0.4 ml of medium = 1:81
- 0.2 ml of 1:81 serum dilution plus 0.4 ml of medium = 1:243

Discard 0.2 ml from last tube so that each tube contains 0.4 ml.

3. For serum-virus mixtures add:

- 0.4 ml virus to 0.4 ml of undil. serum = 1:2
- 0.4 ml virus to 0.4 ml of 1:3 serum = 1:6
- 0.4 ml virus to 0.4 ml of 1:9 serum = 1:18
- 0.4 ml virus to 0.4 ml of 1:27 serum = 1:54
- 0.4 ml virus to 0.4 ml of 1:81 serum = 1:162
- 0.4 ml virus to 0.4 ml of 1:243 serum = 1:486

4. Virus controls are prepared as follows:

- 4.5 ml diluted virus containing 100 TCID$_{50}$ is considered = 10$^{-0}$
- 0.5 ml of 10$^{-0}$ virus plus 4.5 ml media = 10$^{-1}$
- 0.5 ml of 10$^{-1}$ virus plus 4.5 ml media = 10$^{-2}$
- 0.5 ml of 10$^{-2}$ virus plus 4.5 ml media = 10$^{-3}$

Shake all mixtures well and incubate in refrigerator at 4°C for two hours.

5. Inoculate a group of three tubes of bovine kidney cells, each with 0.2 ml of a serum-virus mixture and a group of six tubes each with 0.1 ml of control virus dilution. Six uninoculated tubes are retained as tissue cell control tubes. Incubate tubes at 35-36°C.

6. The test is read three to four days after incubation. Control tubes should have a good monolayer of intact cells but, if spontaneous degeneration has occurred, the test should be discarded. The virus controls are considered satisfactory when the titer is approximately TCID$_{50}$ 10$^{-2}$. Any degeneration characteristic of virus (see figure 2) is regarded as negative (no neutralization). The titer of a serum is calculated by method of Reed and Muench. For a simple test of immunity, undiluted cattle serum only is tested. If no cytopathogenic effect occurs, the animal is considered immune.
Effects of several cattle viruses on bovine tissue-cultured kidney cells. Hematoxylin and Eosin preparations.
INFECTION BoVINE RHINOTRACHEITIS—INFECTION BoVINE PustULAR VulVOVAGINITIS

Virus Neutralization Test

**Materials.** Virus for preparing a stock of virus can be obtained from any laboratory studying this infection.

Bovine kidney cells are used and serums obtained in a manner similar to that for virus diarrhea.

**Procedure.**
1. From titrated stock virus (usually $10^{-6}$) calculate the dilution necessary to make 100 TCID$_{50}$ per 0.1 ml. Dilutions are made with tissue culture maintenance fluid.
2. Serum dilutions are prepared like virus diarrhea.
3. Serum-virus mixtures, virus controls and uninoculated tubes are prepared, inoculated, and incubated in the same manner as for virus diarrhea.
4. The test is read six to seven days after incubation. It is considered a satisfactory test if the virus control tubes indicate adequate virus and uninoculated control tubes show intact cells. Any sign of degeneration characteristic of this virus (see figure 3) is regarded as negative (no neutralization). Tubes failing to show degeneration are recorded as positive (neutralization). Endpoints are calculated by method of Reed and Muench. If undiluted serums from cattle neutralize the virus, they are classified as immune animals.

Hemagglutination Test for Parainfluenza 3 Virus (31)

**Materials.** Stock virus of known hemagglutinating titer should be available. This stock virus is produced in bovine kidney cells maintained with Parker 199 + 0.5 percent lactalbumin hydrolysate (figure 4).

Cattle serums are prepared and handled like virus diarrhea.

Guinea pig red blood cells are collected in Alserver's solution and stored in refrigerator. Prior to use, wash cells three times with PSS solution (.85 percent NaCl) at pH 5.4-6.5. A final suspension of 0.4 percent guinea pig RBC (1:250) is made.

**Procedure.**
1. From titrated stock virus (usually 160 HA units per 0.25 ml), calculate the dilution containing 4 HA units in 0.25 ml using PSS as diluent.
2. To reduce the incidence of nonspecific positive reactions, serums should be treated with acid washed kaolin (Fisher Chemical) and guinea pig RBC. Prepare a stock suspension containing 25 gms of kaolin in 100 ml of PSS. To each tube containing 0.3 ml of undiluted test serum and 1.2 ml of PSS, add 1.5 ml of kaolin stock solution. Mix by inverting tubes three times during the 20 minute incubation period at room temperature. Centrifuge at 1,500 r.p.m. for 10 minutes and retain supernatant. Then add 0.2 ml of 50 percent guinea pig RBC suspension to supernatant and place in refrigerator for one hour. Shake mixture
three times during this period. After one hour centrifuge again at 1,500 r.p.m. for 10 minutes. Pour off and use serums within 24 hours after final treatment.

3. Make twofold dilutions of serum in the test.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25 ml undiluted treated serum = 1:10</td>
</tr>
<tr>
<td>2</td>
<td>0.25 ml undiluted treated serum plus 0.25 ml PSS = 1:20</td>
</tr>
<tr>
<td>3</td>
<td>0.25 ml 1:20 serum plus 0.25 ml PSS = 1:40</td>
</tr>
<tr>
<td>4</td>
<td>0.25 ml 1:40 serum plus 0.25 ml PSS = 1:80</td>
</tr>
<tr>
<td>5</td>
<td>0.25 ml 1:80 serum plus 0.25 ml PSS = 1:160</td>
</tr>
<tr>
<td>6</td>
<td>0.25 ml 1:160 serum plus 0.25 ml PSS = 1:320</td>
</tr>
<tr>
<td>7</td>
<td>0.25 ml 1:320 serum plus 0.25 ml PSS = 1:640</td>
</tr>
<tr>
<td>8</td>
<td>0.25 ml 1:640 serum plus 0.25 ml PSS = 1:1280</td>
</tr>
<tr>
<td>9</td>
<td>0.25 ml 1:1280 serum plus 0.25 ml PSS = 1:2560</td>
</tr>
<tr>
<td>10</td>
<td>0.25 ml 1:2560 serum plus 0.25 ml PSS = 1:5120</td>
</tr>
</tbody>
</table>

Remove 0.25 ml from last tube so that each tube contains 0.25 ml.

4. To each of above tubes add 0.25 ml of virus containing 4 HA units.

5. Make twofold dilutions of virus for controls.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25 ml diluted virus containing 4 HA units = 4 units</td>
</tr>
<tr>
<td>2</td>
<td>0.25 ml of 4 unit virus plus 0.25 ml PSS = 2 units</td>
</tr>
<tr>
<td>3</td>
<td>0.25 ml of 2 unit virus plus 0.25 ml PSS = 1 unit</td>
</tr>
<tr>
<td>4</td>
<td>0.25 ml of 0 unit virus plus 0.25 ml PSS = -1 unit</td>
</tr>
</tbody>
</table>

Discard 0.25 ml from tube four, so that each virus control contains 0.25 ml. Then to each add 0.25 ml of PSS in place of serum.

6. A serum control tube for each test serum is recommended. To a tube add 0.25 ml of undiluted treated serum, then add 0.25 ml PSS instead of virus.

7. One RBC control is needed, so place 0.5 ml of PSS in a single tube to which 0.25 ml are added later.

8. Shake mixtures well and incubate in ice box for one hour. Then add 0.25 ml of guinea pig RBC suspension to every tube.

**Reading the test.** Incubate in refrigerator overnight or at room temperature for one and one-half hours, then read the test. It is read in the same way as the standard hemagglutination test for Newcastle or influenza viruses. Clumping of red cells in the bottom of the tube means that hemagglutination has occurred and the test is negative (no neutralization). A button of cells in the bottom of the tube denotes a positive test (neutralization). Properly adsorbed negative cattle serums usually have titers of 10 or less, whereas, cattle recovered from this infection have titers greater than 40 and range to 2,560.
COMPLETION FIXATION TEST FOR M. Bovis

Materials. Antigen must be prepared and titrated since it is not available commercially as yet.

Antigen is prepared from yolk sacs heavily infected with M. bovis virus by the method described for rickettsial antigens. To a 10 percent suspension in saline of yolk sacs, an equal volume of diethyl ether is added. The aqueous phase is separated after shaking at intervals, centrifuged at 1,500 r.p.m. for 20 minutes to remove tissue fragments, and then vacuum is applied to remove residual ether. A normal yolk sac antigen is prepared in the same way. Antigen titrations are made with a 1:10 dilution of sera pooled from five convalescent guinea pigs. Two units of complement, two units of amboceptor, and two percent sheep cells are used in the test. Each component of the test is employed in 0.2 ml amounts. Guinea pig sera is inactivated at 56 C for 30 minutes and bovine sera is heated at 60 C for 30 minutes. Preliminary incubation is done at 4 C for 18 hours, the hemolytic system added and then incubated again for 30 minutes in a 37 C water bath. The highest dilution of any antigen that shows fixation of complement is considered one unit of antigen.

Cow serum can be procured and prepared in the usual manner. Complement can be obtained from guinea pigs or purchased. It must be titrated for activity and standard laboratory procedures are used. Amboceptor can be prepared or purchased. Sheep red blood cells can be prepared and stored in Alsever's solution for several months.

Procedure. 1. Twofold dilutions of bovine sera are prepared after inactivation at 60 C for 30 minutes.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>0.2 ml undil. serum</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:4</td>
<td>0.2 ml 1:2 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:8</td>
<td>0.2 ml 1:4 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:16</td>
<td>0.2 ml 1:8 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:32</td>
<td>0.2 ml 1:16 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:64</td>
<td>0.2 ml 1:32 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:128</td>
<td>0.2 ml 1:64 dil.</td>
<td>0.2 ml saline</td>
</tr>
<tr>
<td>1:256</td>
<td>0.2 ml 1:128 dil.</td>
<td>0.2 ml saline</td>
</tr>
</tbody>
</table>

Discard 0.2 ml 1:256 leaving 0.2 ml in last tube.

2. To each tube, add 0.2 ml antigen containing 2 units. Then, add two units of complement. There should be control tubes for anticomplementary effects of antigen, sera and dilutions of complement to indicate two units.

Mix and incubate at 4 C for 18 hours.

3. Add indicator system consisting of 0.4 ml of mixture of equal parts of two percent sheep cells and two units of amboceptor. Mix and incubate for 30 minutes in 37 C water bath. Note dilution that completely fixes complement by failure of tube to show hemolysis.
4. If control tubes show sufficient complement and no anticomplementary effects from serum and antigen, then any fixation of complement is indicative of infection.

AGGLUTINATION-LYSIS TEST FOR *Leptospira pomona*

*Materials.* Cultures of *Leptospira pomona* are maintained in Stuart's modified medium for use as antigen.

*Procedure.*

1. Cultures are incubated at 30 C for seven days and held at room temperature for at least 14 days before use as antigens.

2. Dilutions of serum are made in a spot plate. Designate rows A, B and C.

<table>
<thead>
<tr>
<th>Row</th>
<th>Volume of Serum</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.05 ml</td>
<td>1:5</td>
</tr>
<tr>
<td>B</td>
<td>0.06 ml 1:5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.02 ml 1:15</td>
<td>1:15</td>
</tr>
<tr>
<td></td>
<td>0.02 ml 1:5</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>0.06 ml 1:50</td>
<td>1:150</td>
</tr>
<tr>
<td></td>
<td>0.06 ml 1:500</td>
<td>1:1500</td>
</tr>
<tr>
<td></td>
<td>0.06 ml 1:5000</td>
<td>1:15,000</td>
</tr>
</tbody>
</table>

3. Add 0.06 ml of antigen to each dilution of serum in rows B and C. Final dilution of serum is thereby increased twofold. Mixtures are then incubated at 30 C for at least 30 minutes and readings completed within three hours after mixing.

4. *Reading Test:* One drop of mixture is removed to clear glass slide and examined under a dark-field microscope with 10X magnification. Titer is determined by examination for the greatest dilution that agglutinates and lyses leptospira.

The agglutination-lysis test as reported here indicates only the presence or absence of leptospirosis antibodies. A positive test indicates vaccination or a leptospirosis infection which occurred at least seven days prior to obtaining the blood sample. Active disease might have occurred many months before, perhaps unnoticed, inasmuch as antibodies in this disease are known to persist for years.

Leptospirosis testing has now reached a stage where something must be done to differentiate between acute reactions, vaccination reactions, and old declining titers. Beginning October 1, 1959, all positive cattle samples will be titered with a titer of 1:1000 or greater considered as significant.

*Test procedure as done by Dr. T. F. Benson, N. Y. State Diagnostic Laboratory, Cornell University, Ithaca, N. Y.
Serological confirmation of acute leptospirosis depends on obtaining a blood sample at the time of acute illness (when the test is negative), and another sample at least seven to 10 days later (when the test is positive). The fact that the test changes from negative to positive during the period between tests indicates leptospiral infection.

NEED FOR VACCINATION

The primary purpose of livestock vaccination is to increase the efficiency of animal production through prevention of disease. As such, vaccination of livestock is a farm management practice that compares with the prevention of disease in other farm crops, and must be subjected to comparable objective economic appraisal. An economic evaluation of the need for vaccination against a particular disease must ultimately be reduced to a comparison of farm profit which would be realized with vaccination and without vaccination. In an agricultural region free from the specific disease, vaccination could, of course, only reduce the total farm profit. In a region where the disease is present, vaccination may or may not increase the total farm profit. This would depend upon the prevalence of the disease, its degrading effect on livestock, and the cost of vaccination. If the total monetary loss which the region would incur from the disease without vaccination exceeds the total cost of vaccinating all livestock, then vaccination would be an economically efficient management practice. When this comparison is expressed as the ratio of total loss to total cost, the following formula (32) evolves for determining the economic need for vaccination:

\[
\frac{\text{Total loss}}{\text{Total cost}} = \frac{\text{no. diseased animals} \times \text{aver. loss per diseased animal}}{\text{incidence of disease} \times \text{aver. loss per diseased animal}}
\]

This formula effectively points out the three essential factors, disease incidence, average loss per diseased animal, and cost per vaccination, which objectively determine the need for vaccination and which, therefore, must be known in order to give economic justification to vaccination as a farm management practice:

Incidence of disease. Present incidence statistics are compiled primarily from reports by veterinary practitioners and are based, therefore, on clinical diagnoses. Clinical diagnosis, however, is in turn influenced by previously reported incidence. The disease, infectious bovine rhinotracheitis, for example, is well known as a western feedlot disease and is almost never reported in the eastern United States; in recent serum tests of 56 dairy herds in New York, however, 11 of the herds contained reactors (14, 33), suggesting that despite the rareness of reported cases the disease is not uncommon in the dairy herds of this section.
Serological tests represent a completely objective approach to the incidence problem, and when incorporated into a statistically designed survey can be made to produce reliable estimates of disease incidence for any agricultural region. Such a serological survey has been activated in New York with the help of veterinary practitioners throughout the state. A sampling plan (34) was drawn up (Table 1) to obtain two serum samples from each of 500 herds distributed among the counties in proportion to their dairy cow populations. The aid of veterinarians in collecting these samples is being solicited at sectional veterinary meetings, where veterinarians are informed of the purpose of the survey and given brief instructions on the selection of sample herds, the selection of cows within a herd, and the disposition of the serum samples. The response has been enthusiastic, giving every indication that cooperation with veterinarians is a practicable means of obtaining samples.

The serum samples being mailed in to the Veterinary Virus Research Institute at Cornell are being tested for agents of five diseases, leptospirosis infectious bovine rhinotracheitis, virus diarrhea, shipping fever, and Miyagawanaella bovis infection. A statistical evaluation of the sampling plan indicates that the state incidence estimates for these five disease agents will be accurate to within three or four percent, which should be adequate for the purpose of evaluating the economic need for vaccination. The incidence figures obtained will actually estimate the average incidence per herd in the state rather than the percent of diseased animals in the total population of the state. Estimation of the latter would require each cooperating veterinarian to determine herd size for those herds which he samples, and this was considered too great an imposition on him. Insofar as herd incidence is independent of herd size, however, these two incidence figures will be equal.

The experience with this serological survey plan in New York State indicates that it could be conducted on a national scale, with or without the supervision and sponsorship of a central agency. A national plan has been devised (35) and the New York State survey was inaugurated, in part to test the practicability of this plan. No central agency has yet moved to undertake the survey, however, so national coverage can only be obtained on a state by state basis. A sampling plan similar to that for New York State, could be devised for each interested state, and the state's own serological laboratory used to test their serum samples.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allocation of 1,000 Milk Cow Serum Samples to 54 Counties</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>County</th>
<th>1964 Population Farms</th>
<th>1964 Population Cows</th>
<th>Proposed Sample Farms</th>
<th>Proposed Sample Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>695</td>
<td>11,109</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Allegany</td>
<td>1,756</td>
<td>24,920</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Broome</td>
<td>1,440</td>
<td>23,567</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Cattaraugus</td>
<td>2,447</td>
<td>45,153</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Cayuga</td>
<td>1,449</td>
<td>23,060</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Chautauqua</td>
<td>2,915</td>
<td>42,141</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Chemung</td>
<td>732</td>
<td>8,656</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>
### TABLE 1 (continued)

<table>
<thead>
<tr>
<th></th>
<th>1954 Population</th>
<th>Proposed Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farms</td>
<td>Cows</td>
</tr>
<tr>
<td>8</td>
<td>Chenango</td>
<td>1,878</td>
</tr>
<tr>
<td>9</td>
<td>Clinton</td>
<td>1,588</td>
</tr>
<tr>
<td>10</td>
<td>Columbia</td>
<td>904</td>
</tr>
<tr>
<td>11</td>
<td>Cortland</td>
<td>955</td>
</tr>
<tr>
<td>12</td>
<td>Delaware</td>
<td>2,469</td>
</tr>
<tr>
<td>13</td>
<td>Dutchess</td>
<td>824</td>
</tr>
<tr>
<td>14</td>
<td>Erie</td>
<td>2,169</td>
</tr>
<tr>
<td>15</td>
<td>Essex</td>
<td>641</td>
</tr>
<tr>
<td>16</td>
<td>Franklin</td>
<td>1,415</td>
</tr>
<tr>
<td>17</td>
<td>Fulton</td>
<td>521</td>
</tr>
<tr>
<td>18</td>
<td>Genesee</td>
<td>1,150</td>
</tr>
<tr>
<td>19</td>
<td>Greene</td>
<td>777</td>
</tr>
<tr>
<td>20</td>
<td>Herkimer</td>
<td>1,445</td>
</tr>
<tr>
<td>21</td>
<td>Jefferson</td>
<td>2,547</td>
</tr>
<tr>
<td>22</td>
<td>Lewis</td>
<td>1,372</td>
</tr>
<tr>
<td>23</td>
<td>Livingston</td>
<td>1,089</td>
</tr>
<tr>
<td>24</td>
<td>Madison</td>
<td>1,532</td>
</tr>
<tr>
<td>25</td>
<td>Monroe</td>
<td>1,172</td>
</tr>
<tr>
<td>26</td>
<td>Montgomery</td>
<td>1,077</td>
</tr>
<tr>
<td>27</td>
<td>Niagara</td>
<td>1,522</td>
</tr>
<tr>
<td>28</td>
<td>Oneida</td>
<td>2,618</td>
</tr>
<tr>
<td>29</td>
<td>Onondaga</td>
<td>1,485</td>
</tr>
<tr>
<td>30</td>
<td>Ontario</td>
<td>1,401</td>
</tr>
<tr>
<td>31</td>
<td>Orange</td>
<td>1,281</td>
</tr>
<tr>
<td>32</td>
<td>Orleans</td>
<td>827</td>
</tr>
<tr>
<td>33</td>
<td>Oswego</td>
<td>1,963</td>
</tr>
<tr>
<td>34</td>
<td>Otsego</td>
<td>2,375</td>
</tr>
<tr>
<td>35</td>
<td>Putnam</td>
<td>124</td>
</tr>
<tr>
<td>36</td>
<td>Rensselaer</td>
<td>1,114</td>
</tr>
<tr>
<td>37</td>
<td>St. Lawrence</td>
<td>3,816</td>
</tr>
<tr>
<td>38</td>
<td>Saratoga</td>
<td>1,178</td>
</tr>
<tr>
<td>39</td>
<td>Schenectady</td>
<td>284</td>
</tr>
<tr>
<td>40</td>
<td>Schoharie</td>
<td>1,208</td>
</tr>
<tr>
<td>41</td>
<td>Schuyler</td>
<td>579</td>
</tr>
<tr>
<td>42</td>
<td>Seneca</td>
<td>578</td>
</tr>
<tr>
<td>43</td>
<td>Steuben</td>
<td>2,397</td>
</tr>
<tr>
<td>44</td>
<td>Suffolk</td>
<td>313</td>
</tr>
<tr>
<td>45</td>
<td>Sullivan</td>
<td>967</td>
</tr>
<tr>
<td>46</td>
<td>Tioga</td>
<td>1,130</td>
</tr>
<tr>
<td>47</td>
<td>Tompkins</td>
<td>927</td>
</tr>
<tr>
<td>48</td>
<td>Ulster</td>
<td>864</td>
</tr>
<tr>
<td>49</td>
<td>Warren</td>
<td>537</td>
</tr>
<tr>
<td>50</td>
<td>Washington</td>
<td>1,717</td>
</tr>
<tr>
<td>51</td>
<td>Wayne</td>
<td>1,364</td>
</tr>
<tr>
<td>52</td>
<td>Wyoming</td>
<td>1,567</td>
</tr>
<tr>
<td>53</td>
<td>Yates</td>
<td>687</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>71,582</td>
<td>1,292,887</td>
</tr>
</tbody>
</table>
Average loss per diseased animal. The problem of determining the average monetary loss in productivity per diseased animal can be approached in several ways. Perhaps the most direct approach is the use of controlled experiments, in which susceptible animals are challenged with the infectious agent, and their resulting loss in productivity measured by comparison with the productivity of vaccinated control animals. An alternative approach, a modification of this experiment to field conditions, consists of studying herds in the field by vaccinating half of each herd and administering a placebo to the other half; natural infections in the placebo group should then produce a measurable loss in production as compared to the vaccinated group. Such a preliminary study is now underway at the Institute, using infectious bovine rhinotracheitis vaccine in 13 dairy herds of central New York; productivity, in this case, is measured by the milk production records.

Cost per vaccination. The cost of vaccination to an individual owner equals the price of the vaccine plus the cost of administering it—which includes both the veterinarian’s professional fee and the owner’s labor costs for assembling and handling the animals to be vaccinated. Accurate assessment of these costs presents no problem; rather, we wish to point out the possibility of reducing these costs through the use of combined vaccines (36), thereby increasing the economic efficiency of vaccination as a management practice.

The cost of the vaccine is determined in part by such expenditures as potency testing, production, and sales promotion. Clearly, these commercial expenditures for a combined vaccine would be lower than those of several vaccines sold separately. The other component of the cost of vaccination—the practitioner’s fee and owner’s labor costs—would be the same for a combined vaccine as for each single vaccine; the owner would thereby obtain protection for his livestock against several diseases for merely the additional cost of the vaccine.

The economic value of a combined vaccine depends, of course, upon the same factors as were described for a single vaccine. An objective evaluation and, in fact, the choice of the individual components of a combined vaccine require accurate information about the incidence and effects of the disease and cost of vaccination. Conceivably, situations would arise in which a given vaccine, administered singly, would not be economically efficient, but would be efficient if administered in combination with others, because of the slight added cost of the vaccine alone.

STANDARDIZATION OF VACCINES

Once the need for vaccination is established on an economic basis, livestock producers may be expected to respond through their veterinarians with a demand for a commercial vaccine. In order to fulfill its economic objective, of course, the commercial vaccine must be safe and effective; that is, it must produce no significant illness, and it must immunize. In order to guarantee these features in all commercial vaccines, government control must be exerted in the form of acceptability standards.
Ideally, no vaccine should be sold which will ever produce serious illness or will ever fail to immunize a susceptible animal. In reality, however, no amount of testing could ever prove with certainty either of these properties, since there is always the possibility that the next animal tested will produce a failure. Because of the natural variation in biological systems, no biological product can actually be expected to be perfect. Standards, therefore, are forced to allow for this uncertainty and imperfection, and hence must be formulated on statistical grounds. In this respect, vaccine standards have a common ground with acceptability standards used in all fields of government and industry to control product quality. Since statistical techniques for testing acceptability of a product have been highly developed by the purchasing agencies of industry and government, especially the military branches of the government, some of these advanced techniques and principals may profitably be incorporated into a modernized set of standards for vaccines. In detail, however, vaccine standards must depend intimately on the properties of viral agents and the process of vaccine development. Standards, therefore, will be described with reference to the several steps involved in the development of a vaccine as shown in figure 5.

**Safety and efficacy test at the lowest passage level to be used in vaccine.** Safety in a live virus vaccine has two aspects: The attenuated virus must produce no signs of illness, or agreed upon modified signs of illness, and if the vaccine virus is transmissible by contact exposure then there must be no reversion to virulence after five continuous passages. Since animals reared together are ordinarily vaccinated together, the chances of five passages actually occurring under field conditions are believed to be extremely small. The attenuated virus continues to decrease in virulence with successive passages through the alien host; consequently, it is necessary and sufficient that safety be demonstrated for the lowest passage level to be used in the commercial vaccine.

Efficacy, on the other hand, tends to decrease with further attenuation of the vaccine virus; it is therefore necessary and sufficient that efficacy be demonstrated for the highest passage level to be used in vaccine. Because of the nature of the safety test, however, efficacy also can be tested at the lower passage level with no additional effort and thus provide the producer with a preliminary efficacy check. If the vaccine virus fails to pass the efficacy test at the lowest passage level it would almost certainly fail at any higher passage levels, and the producer would, therefore, have to go back to an even lower range of passage levels to test for commercial use.

Safety, and also efficacy, can be ascertained only in animals which are susceptible at the time of vaccination. An immune animal will, of course, show no signs of illness in response to vaccination, no matter how virulent the vaccine virus may be, nor will he transmit the vaccine virus to a contact animal. Ideally, therefore, all animals to be used in the test should be susceptible. This can be accomplished with certainty only if a serological test for immunity is available, and then immune animals should be culled out at the time of vaccination.
FIG. 5. Steps in the Development of a Commercial Vaccine

**Virulent virus**

Produces illness and productivity loss in natural host.

\[ \downarrow \]

**Serial passage in alien host**

\[ \downarrow \]

**Attenuated virus**

Modified effect in natural host: Produces no signs of illness or produces modified, acceptable signs of illness.

\[ \downarrow \]

**Preliminary safety test**

Test of attenuated virus in natural host for signs of illness and transmissibility; if transmissible, then number of passages for reversion to virulence must exceed 5.

\[ \downarrow \]

**Continued passage in alien host to highest level**

\[ \downarrow \]

**Determination of minimal immunizing dose**

Immune response to dosages at the highest passage level to be used.

\[ \downarrow \]

**Laboratory standardization of vaccine**

Determination of ID₅₀ for each batch to eliminate batch differences in the commercial dose.

\[ \downarrow \]

**Efficacy test and safety check**

Efficacy field tested for acceptability at the highest passage level to be used in vaccine; vaccinated animals observed for signs of illness due to vaccination and checked for immunity in a paired challenge test or unpaired serological test. Repeated annually to assure continued quality.

The design of the experiment for testing safety (and efficacy) in susceptible test animals is borrowed, in principle, from industrial acceptance sampling plans (37) because of the efficiency of these plans. Paired animals, a vaccinate and a contact control, are tested in sequence until the rules of the experiment dictate one of the following decisions: (a) stop the paired animal experiment and conclude that the vaccine is unsafe because the vaccine virus is too virulent, or (b) stop the paired animal experiment and conclude that the vaccine is safe because it does not produce undue illness and does not transmit to contact controls, or (c) stop the paired animals experiment
VACCINATION OF CATTLE FOR INCREASED PROFITS

because the vaccine virus is transmitted to contact controls, but continue testing vaccinates for signs of illness until a decision regarding that aspect of safety is reached and, at the same time, begin three series of five passages each through susceptible animals to measure reversion to virulence. The detailed rules of this acceptance test, shown in figure 6 are constructed so as to guarantee with practical certainty (probability greater than 95 percent) that any vaccine which is at least 90 percent safe will be accepted and any which is less than 90 percent safe will be rejected. Notice that a minimum of 45 vaccinates is required to show safety, and then only if all 45 show no undue signs of illness.

The experimental animals in the safety test should be kept under controlled laboratory conditions; each vaccinate and its control should be kept in an isolation pen to eliminate the confounding effect of other diseases. All animals should be tested serologically three to four weeks after vaccination and challenged at the same time with virulent virus to confirm and refine the serological test for immunity. The vaccinates should pass the sequential efficacy test shown in figure 6. Transmissibility will have been demonstrated as soon as one contact control develops an immune response, and from then on vaccinates may be used without contact controls.

If no serological test is available to pick out susceptible animals for the safety test, then three series of animals are required for the experiment; the two series of vaccinates and contact controls must now be supplemented by a third series of isolated controls to measure the natural immunity rate in the test animals. All animals coming from a common source should be randomly allotted to three groups to assure that, outside of random sampling error, this natural immunity rate is constant within the three series. Animals immune at the time of vaccination, of course, contribute no information with respect to the safety (or efficacy) of the vaccine, and this is reflected in the rules of the sequential acceptance test in that the decision to accept or reject depends upon the ratio of the number of vaccinates showing undue signs of illness to the number of susceptible control isolates. Susceptibility is measured only by direct challenge with virulent virus when no serological test is available, and efficacy is tested as shown in figure 7 by comparing the number of susceptible control isolates. As soon as the number of contact controls showing immunity exceeds the upper bound in figure 7 then, again, the contact control series can be terminated in the test and the three series of five passages each initiated to test reversion to virulence.

Determination of the minimal immunizing dose. A minimal immunizing dose of the modified virus at the highest passage level to be used in vaccine should be calculated by the Reed-Muench formula using three animals at each of four dosage levels. Their dose is to be related to a titrated number of laboratory tested units; a laboratory test may be defined as making use of laboratory animals, embryonated eggs, tissue-cultured cells or other acceptable methods.

This minimal dosage then must be multiplied by a factor sufficiently large to assure an amount of virus considered ample for a commercial dose, which then is to be tested for efficacy in animals similar to those for which the
vaccine is being produced, according to the statistical design described below. Batch to batch differences will be effectively eliminated by routine laboratory assays of the amount of virus present, so that commercial doses will always contain the same amount of virus, regardless of the batch.

Test of efficacy at the highest passage level to be used in vaccines. If possible, the efficacy of a vaccine at the highest passage level should be tested in the field by a government control agency. This can be done conveniently only if an accurate serological test for immunity is available; the serum samples before and after vaccination can then be assayed in a government laboratory. The efficacy test, in this case, would not require that the vaccinates be challenged with the virulent virus since the relationship between immunity and antibody titer would have been previously established. Post-vaccination serum samples would be obtained only from animals which tested susceptible in the pre-vaccination sample, and the serum tests would continue until a decision to accept or reject the vaccine is reached according to the rules in figure 6. Safety should, of course, be checked by noting any undue signs of illness in the vaccinates, but transmissibility need not be measured; if it occurs it can only serve to increase the efficacy of the vaccine. This field test of efficacy should be repeated annually to guarantee continued quality of the produce.

Fig. 6

![Diagram](image)

**Fig. 6.** Rule for a sequential acceptance test using paired susceptible vaccinate and contact control animals.

If no serological test for immunity is available, then a control series, as well as a vaccinate series, are required for efficacy tests, and if the vaccine virus is transmissible by contact, then the controls must be kept isolated from
the vaccinates. Since immunity is measured by challenge with virulent virus in this case, the experiment cannot be conveniently conducted in the field. The decision to accept or reject the vaccine is determined by the rules in figure 7.

**Fig. 7**

![Diagram](image)

Fig. 7. Rule for a sequential acceptance test using triplets of vaccinate, contact control and isolated control animals of unknown immunity status.

**CONCLUSIONS**

An assay of loss from any disease can be made if there is a serological test to determine incidence. A figure can be obtained simply by multiplying the number of animals affected by the average loss per animal, whether loss be due to death, decreased milk production, abortion or depressed weight gains. To make such an effort worthwhile, loss must be prevented so that an amount of dollars, less the cost of prevention, can be added to profits. Vaccination offers the best means of doing this for a number of recently studied diseases of cattle, especially leptospirosis, IBR-IPV and virus diarrhea. Furthermore, a combination of immunizing products for these three diseases can be made into a single vaccine. A combination should be cheaper to produce and to use and, thereby, enhance the total economic benefit to be derived from vaccination.

A vaccine must be safe and effective. Simplified standards to accomplish this are suggested. Furthermore, it is proposed that this combined vaccine, or any other, should be introduced as a measure to increase productivity, rather than offering another disease preventative in the usual manner.
REFERENCES


Vertical integration or so-called contract farming has developed in various areas in several states. An explanation of integrated livestock production or contract animal production is as follows.

Horizontal integration is the combination of livestock productions that are alike into one larger business. In livestock production an example of this would be when two farms are combined under one management. Vertical integration is the combination or centralization of two or more of the various stages of production, processing and marketing of a product under one management. Dairy cow pools, broiler production, contract beef cattle feeding and large hog enterprises are examples of this type of livestock production.

Vertical integration has been favored to date by large feed companies, although other allied industries have been involved. The broiler industry is 95 percent integrated, with feed companies acting as integrators. Feed, medicine, vaccines, fuel and birds are furnished by the contractor, while the farmer supplies the housing and labor. The title of the chicks remains with the contractor while the grower (the farmer) receives a flat fee, profit split or bonus for his labor and buildings. The broiler industry, as a result, has grown from a production of 300 million in 1946 to 1.4 billion in 1958, with the price going from 30 cents to 15 cents a pound.

It is estimated that all of the livestock production enterprises are partially integrated with percentages as follows.

- Cattle — 10-20 percent
- Lambs — 25 percent
- Hogs — 5-10 percent
- Broilers — 90 percent
- Turkeys — 75 percent
- Market Eggs — 10 percent
- Milk — 5 percent
- Hatching Eggs — 95 percent

The biggest development since World War II is the growth of commercialized feedlots which feed from 1,000 to several thousand head of cattle in a continuous operation. Larger feeders are able to mobilize feed resources from a larger area. Colorado, Arizona and California lead in commercial beef feeding on a contract basis.

In the swine field it is estimated that swine production is moving to the south from the hogbelt; moving to areas of cheaper labor and where feed tonnage is desired. However, scattered throughout the country are hog enterprises of considerable size, some integrated, some not, but with production up to 100,000 pigs a year. Several states likewise are gearing themselves to make their pork consumption self-produced. Pennsylvania, California and Florida are attempting to do this in the next few years.

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The sheep industry has experienced contract feeding for several years. However, it is not a consistent factor, dependent upon market fluctuations for encouragement.

Turkeys: A majority of the turkey production in the United States is on a contract or integrated basis. In most all cases the feed industry is tied to this phase of integration.

In the dairy business large units have not been uncommon on the west coast. However, in the midwest and in the east smaller herds have predominated. Recently a young enterprising Iowan developed a cow pool in which he organized and implemented a contract milking set up for 1,000 cows. A number of adversities plagued him at the start in the form of poor management with the lack of disease control facilities as his biggest problem. Basically, he contracted for farmers’ cows, in many cases entire herds, without any health supervision. In a few months his problems mounted to the point that he obtained veterinary service, cleaned up his pool and is now running a successful business. However, at the start his “pool” received national interest and at one time over 40 different pools were in the planning stage. To date cow pools exist in Iowa, Kansas, Oregon and several other states. There will be more in the future.

The basic fundamental problem with contract feeding, milking or assembly of large groups of animals is disease control. The first responsibility of the contractor is to have a health program. Usually with some encouragement he will seek veterinary advice. Thus it falls in the hands of the attending veterinarian to outline a preventive health program. In some types of contract livestock production ownership of the animals does not change, thus State Regulatory people may not always have control over the movement of livestock. This can cause serious disease problems and presents greater problems if disease does break out. This was handled in the cow pool in Iowa by requiring that all animals entering the pool be inspected at their point of origin by the dairyman’s veterinarian who is consigning the cows. Then when the cows entered the pool they were quarantined and isolated for thirty days before they entered the main milking line-up.

Basically, the problem of cow pools or other types of contract farming is not one of failing to comply with existing disease control programs, but the problem of getting together many animals in one concentration. Health, sanitation and management take on major importance when cattle are assembled in large numbers from many sources. Diseases that normally may not cause financial losses in a small herd can readily create havoc in a large herd.

One of the greatest problems in the concentration of animals is the manure removal problem, plus the fly population that exists in such areas. These are problems that not only health authorities but animal disease regulatory personnel may be called upon for advice and enforcement.

Veterinarians associated with contract livestock production either as disease control specialists or as business men should keep in mind the following fundamental points and should study them closely.
1. Period of contract: The period of the contract should be certain. Both starting and termination dates should be spelled out.

2. Renewal provisions: Some contracts call for automatic renewal. All parties in the contract should have the same privileges.

3. Cancellation provisions: Few contracts call for cancellation by the livestock producer. There should be equal rights for all parties in the contract.

4. Assignment of interest: Some contracts prevent either party from assigning his duties to another party without written consent. A livestock producer may desire to sublet part of his contract or turn it over to another party. Such provisions should be in the contract.

5. Legal relationship of parties: The livestock producer should know whether he is a farmer, employee or independent contractor. If he is an employee, the integrator may be liable for actions of the farmer that cause injuries or losses to a third party. The same holds true for social security contributions. If the livestock producer was an independent contractor this would not hold true.

6. Supplies furnished by each party: The contract should state specifically items to be furnished by each party.

7. Management decisions: The livestock producer should understand the extent to which the integrator can make management decisions.

8. Producer payments: The contract should state specifically prices, markets and bonus if to be paid for the livestock producer.

9. Arbitration provisions: If disputes arise the contract should contain provisions for settlement without long and costly court procedures.

In summary, vertical integration or contract livestock production as far as disease control actually does not present immediate problems of any magnitude. Large livestock operations, integrated or not, are set up with the idea of efficiency and profit in mind. Thus, sanitation, immunization procedures and adequate veterinary service will be supplied. In most cases disease control practices will be more stringent than what exists on other farms. Also, attending veterinarians in integrated or large specialized operations are given a better chance to practice preventive medicine.
REPORT OF THE COMMITTEE ON DISEASE CONTROL
IN LIVESTOCK INTEGRATION


A questionnaire was prepared by the Committee on Disease Control in Livestock Integration and mailed to the 48 adjoining states.

Through pure oversight Alaska and Hawaii, our newly-admitted states were not included and with a sense of deep humility the members of the Committee wish to offer their sincere apologies.

A total of 40 questionnaires were returned with some very interesting and informative results.

In answer to the question: Do you have any form of livestock integration? 26 states answered in the affirmative, four replied, very limited; and 14 gave a negative reply.

With reference to the classes of livestock involved, eight states reported integration or some ramification thereof involving dairy cattle; eight states reported integrated operations in beef cattle; 14 in swine; five in sheep and 24 reported integration in poultry. Relative to the extensiveness or the size of the operation we received a great variation of replies, ranging from very limited to a maximum of 90 to 100 percent of the total production. The last figure quoted, however, involves integration in poultry production in one of our eastern states.

Of the 26 states reporting integration presently in existence, in one form or another; 12 states reported an estimated total of 11,850 integrated operations. The remaining 14 states reported such information as not available.

The estimated number of animals involved in the average integrated operation again produced a great variation in replies, ranging from a minimum of fifteen large animals per individual operation, to a maximum of two million in poultry. The two million figure in this case involved the integrated broiler industry on a state level, in one of our northeastern states.

The question was asked whether or not integration in livestock was on the increase: and of the 26 states reporting integration presently existing, 13 answered yes, seven no, and six replied that such information was not available. Of the 13 states reporting integration on the increase one eastern state reported the increase as slight, a southeastern state a very definite increase, and a western state reported integration on the increase but proceeding very cautiously.

A question relative to regulatory disease control problems created by integration also produced a variance in opinion.

Eighteen states reported no increase in regulatory problems. In fact, several states reported disease control problems had actually been simplified.

A midwestern state which reported the disease control problems in poultry as having been simplified, because the integrator realized that he must protect his investment; also reported that an integrated endeavor involving
swine was very suddenly disintegrated due to an outbreak of atrophic rhinitis. Following are expressions of opinion from various states and the geographical location of the state reporting.

An eastern state which reported virtually the entire poultry industry as integrated, also reported the disease control program simplified as the result of having fewer individuals with whom to deal.

A southwestern state reported a few cow-pools had been in operation during the past, but were soon discontinued.

A western state replied integration has stimulated agitation for "self-vaccination."

Another western state reported a situation, where all the dairy herds in a community are milked in a common milking parlor, then returned to the individual farm following milking, had not experienced any more difficulty in dealing with this type of operation, than in similar operations where the total cattle population is comparable.

A western state replied that in general it was easier to deal with the management of an integrated firm, than with individuals since they are more interested in preventative measures.

A central plains state reports the only disease problems were those attendant to overcrowding.

On the other side of the ledger two states reported disease control problems arising from integration.

A northeastern state reports with reference to poultry that very few operators have maintained good disease control practices. Many do not have a good personnel, sufficiently trained to do a good job in disease control and sanitation. Lack of sanitation and travel of human carriers are spreading more disease than is spread in any other way.

The other, a midwestern state, reports disease control problems due to variance of health status of individual entry into the integrated operations coupled with the variance of opinion of the common participant to the integrated operation. Some want to do their best to comply—others want to get by, or do without complying if possible.

Two states reported difficulty in establishing herd or flock ownership in conducting state-federal disease control programs, 23 states replied "No."

Thirty-five of the 40 states reporting, have no regulations governing integrated operations, two states answered yes and three did not reply to this question.

One state is at present in the process of writing regulations covering integration; two expressed themselves as undecided; three reported such action would be taken when necessary; eleven did not contemplate such action at present; and thirteen did not reply.

In summarizing it would appear (1) integration presently exists in one form or another in at least 50 percent of the states, involving all classes of livestock, but so far is largely confined to the poultry industry; (2) livestock integration is definitely on the increase but to date very few regulatory disease control problems have been created; (3) a vast majority of the states do not have regulations governing integrated operations in livestock nor do they anticipate promulgating such regulations until the need arises.
THE CALIFORNIA MASTITIS TESTING PROGRAM
IN SHASTA COUNTY

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As one of a team of four, I wish to express our appreciation for the opportunity to discuss with you the California Mastitis Testing program, with especial reference to the pilot county of Shasta.

Recall with me, if you will, that in 1947 California had a mastitis control project, the purpose of which was to control chronic mastitis in dairy cattle caused by Strept. agalactiae. The diagnostic tool was the Hotis test. The goals were:

1. To assist the organization of dairymen, under the sponsorship of the Dairy Herd Improvement Association (or other recognized dairy organization), for the purpose of providing at a reasonable cost:
   a. Diagnosis of mastitis by means of the Hotis test and microscopic study.
   b. Herd management and sanitary milking practices to control spread of mastitis caused by Strept. agalactiae.
   c. Treatment of positive (infected) cattle.

2. Locate and use as demonstration of results typical dairy herds where Strept. agalactiae type of mastitis was being successfully controlled.

In the project we spelled out the responsibilities of the cooperator, the farm advisor, the Extension veterinarian, etc. This project was initiated in several counties in the state. Today San Diego County alone has remained with the project as it was written. In the meantime, a wide variety of antibiotics and other drugs have become available so that a mastitis project is not now confined solely to Strept. agalactiae.

The problem which we faced in the forties has been magnified in the fifties by two factors: prodigious advertising by the antibiotic manufacturing companies, and the ready availability of antibiotics in feed store, drug stores, and even through mail order houses. This has made our problem one of combating "do it yourself" mastitis control in almost every dairy barn throughout the state. This "do it yourself" trend has added a terrific cost to the dairy industry and developed, in many instances, bacteria which can resist these antibiotics.

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When I say terrific cost, I mean just that. The reported 229 million dollar toll that mastitis takes annually in our nation’s dairy industry is a lot of money—even in these inflationary times. And California dairy producers share a big percentage of that loss—estimated at about 35 million dollars.

Furthermore, we must expect, in the near future, that the Federal Food and Drug Administration will consider the outright banning of penicillin in mastitis preparations. Currently, the FDA’s thinking is bolstered by a recent National Research Council report recommending that no tolerance be set for residues in milk. And FDA may start casting suspicious glances at even inactive ingredients of veterinary medications, such as mineral oil.

The dairy industry—nationwide—is in jeopardy, because it IS UNLAWFUL TO SELL MILK CONTAINING ANTIBIOTICS. All right, now we know the problem. What are we going to do about it?

First, let us review the mastitis complex for a moment. We have no other disease of the magnitude of the mastitis complex. The complex comprises 16 to 21 types of bacteria, a variety of fungi, rickettsia, and recently a virus has been incriminated.

We must eliminate the guess work, the “do it yourself-ism”, the confusion, from mastitis control treatments. Twenty-one kinds of bacteria don’t mean we need 21 kinds of control program.

Until recently we were deplorably ignorant about the association of trauma with the mastitis complex. It has taken me some time to accept this particular concept, which has changed my entire viewpoint on mastitis control. Let us explore some of the work on this phase of the mastitis complex.

At the University of California we are using a Strain-Gage amplifier to detect maladjustments, mechanical imbalances and worn-out parts of milking machines and equipment which can cause traumatic injury to teats and udders of lactating cows. A Strain-Gage amplifier, when used in human medicine, discovers circulatory and lymphatic deviations. It can perhaps be best compared to the lie detectors used in police work. When the Strain-Gage amplifier is used to check milking machine equipment, a needle is inserted into the liner tubes which registers the rest phase and the milking phase in the milking process. The phases are permanently recorded on graph paper.

Too little or too much vacuum in the milking system can lead to mastitis. Vacuum pumps influence the total amount of vacuum in the line, the extent of fluctuation in a line, and the number of milking units that can be used. Many vacuum pumps are inadequate and cause excessive drops in the vacuum system as the machines are changed. Other pumps cause pressure vibration in the system. Often, when a change is made from bucket milking to a pipeline system, or when units are added to a system, the vacuum pump is not changed. As a result, many barns are milking with inadequate vacuums.

The construction of the milk barn will influence the height at which pipelines must be installed, the length of the line, and the number of risers, elbows and tees that have to be installed. All these factors influence fluctuating vacuums and the rate of vacuum drop—influencing, in turn, the mastitis rate in a herd.

I am now of the definite opinion that veterinarians, farm advisors, and the servants of the public should encourage dairymen to run monthly Cali-
fornia Mastitis Tests on all lactating cows and prevail upon dairymen to keep individual monthly records. At the end of three months they should have a fairly accurate record of the cows that are consistently positive and those that are consistently negative.

I would then recommend that milk samples from 10 percent of the consistently positive animals be submitted to a veterinarian or diagnostic laboratory for bacterial identification. Further, sensitivity tests should then be run to determine the most efficacious antibiotic or drug or combination thereof for use in the herd—when the cows are in the dry state.

The California Mastitis Test (CMT) is a new procedure for estimating cell content of milk. Irritation to mammary tissue, such as occurs in mastitis, results in an increase in the number of body cells in the milk. Chemical compounds belonging to the group of surface active agents containing long-chain hydrocarbon salts have been found to become visibly altered in the presence of native proteins of cellular origin. Such compounds can be used on milk to detect the increased cell content resulting from mastitis. This is the principle of the California Mastitis Test.

We must not forget this: the CMT is a screening device—not a diagnostic agent per se—and the CMT does not tell us if the inflammation of the udder is of bacterial, rickettsial, viral or traumatic origin.

In California, the Dairyman's Cooperative Creamery Association of Tulare pioneered the CMT program among their shippers. In their program they made a fieldman available to advise shippers regarding malfunctioning milking machine equipment, sanitation and other field management problems. They expanded plant laboratory facilities to run CMT and sensitivity tests for their shippers, which resulted in the upgrading of the quality of the milk received and increased the net return to those shippers who availed themselves of the Association's services. Today some 125 shippers are in the program.

Furthermore, this Association financed the purchase of a Strain-Gage amplifier and made a present of it to the Mastitis Study Committee of the University of California as an additional instrument to further laboratory and field research in mastitis control. The monetary value of this gift to the Mastitis Study Committee and to the California dairy industry is difficult to estimate.

Shasta County, under the leadership of Farm Advisor Francis F. Smith, initiated a CMT program in 14 DHIA herds comprising approximately 1,500 cows. The 14 dairies were shippers to McColl's Dairy Products Company in Redding. At the conclusion of the year there was an over-all gain of two and one-half pounds butterfat per cow per month. Those dairymen who were willing and able to follow the program in the most detail gained considerably more than the average net gain per cow per month. To cite only a few examples:

One cooperator gained one and one-half hours milking time at each milking by correcting maladjusted and malfunctioning milking machine equipment. Another cooperator, after making recommended changes in milking equipment and milking practices, gained $8,500 over the previous year’s return.
For each of the 14 herds, a record was kept on the CMT reactions of each lactating cow each month, on conditions prevailing at the initiation of the program, pulsator patterns, and changes in equipment and procedures. At the end of the first year's program dairymen's comments were summarized. An example is the Walter E. Matthews herd:

**CMT Graph**

![CMT Graph - Matthews Herd](image)

*Conditions prevailing at start of CMT program (March 1957)*

Four stall parlor barn. DeLaval pipeline system with milk pump. DeLaval 05 liners. One man on four units. Vacuum 14 inches.

*Changes in equipment and procedure*

Installed DeLaval 03 liners in April 1957, which resulted in faster milking. Hired a helper in order to keep up with machines. Wipes pulsator piston clean daily. Cleans air release valve in claw daily with acid cleaner. Rinses teat cups in low foam detergent, followed by dip in chlorine type disinfectant after each cow. Changes solutions after 15 cows. Liners are used one week, boiled in lye water and rested one week, then re-used one week. Sponges teats with chlorine solution after milking.

*Dairyman's comments*

Flare-up in January 1958 resulted from dropping the controller weight which caused it to stick, giving rise to erratic vacuum. Wet weather and a new milker aggravated the problem. Attributes 50 pounds butterfat per cow annual production increase largely to reduction in mastitis. Is positive that he was formerly over-milking his cows. Believes that milking machine study deserves priority in University's research. Also feels need for simplified guide for selecting drugs when treatment is necessary.

*Stimulation for Milk Letdown:*

All dairymen recognized the importance of adequate stimulation of the teats prior to milking. Further, all attempted to attach teat cups at the proper time to utilize natural milk letdown to the utmost.
Segregation:
None of the cooperating dairymen segregated his herd for the purpose of milking the "clean" cows first. A system for by-passing the "2" and "3" CMT reactors was set up in the Matthews herd in 1957 but was soon abandoned as impractical and unnecessary. The reduction of mastitis achieved by dairymen in this program indicates that when other deficiencies are corrected, segregation is of little or no importance.

Bacteria Count:
Six dairymen in this study reported a reduction in bacteria count. The other eight stated there was no change, but those eight always had consistently low counts and there was little room for improvement. The evidence seems clear that mastitis problems and high bacteria counts often go hand in hand and that overcoming the former will help correct the latter.

Milk Production:
When possible, the production of each dairyman's herd during the first month of the CMT program was compared with the production during the same month a year later. The average gain was nearly two and one-half pounds of butterfat per cow, or 25 to 30 pounds of butterfat per cow per year. The full impact of the improvement is not felt in one year because the effects of old injuries may prevail for the duration of a lactation or longer.

Milking Time:
There are many ways to relate "milking" and "time." We can list the number of cows, the number of units, the number of operators, and the number of hours in the barn. We can calculate cows per hour per unit, or minutes per unit per cow. None of these figures is very meaningful. From the cow's point of view only one figure is important: how long the machine is attached to the udder after milk flow ceases.

There is ample experience to indicate that when milking equipment is functioning properly and cows are primed properly, most cows will milk out in three to five minutes. Some will require longer, but most fall within the three-to-five-minute category.

Dairymen find that the "light" quarters are usually responsible for the positive CMT reactions. This confirms the premise that over-milking is highly detrimental.

In instances where two different men milked the same cows with the same equipment in the same elapsed time, one man had very little mastitis trouble but the other ran into grief immediately. The difference is attributable to one thing: the length of time the machines were on the cows.

Experience indicates that it is impractical to attempt to set forth the number of units one man should operate. The type of barn, the facilities for feeding, washing, etc., and the speed and habits of the operator tend to create so many variables that no set of specifications will apply universally.

There is only one rule: DON'T OVERMILK.
Milking Ratio:

The milking ratio, or pulsation ratio, is defined as the ratio in time between the vacuum phase and the atmospheric pressure, or rest phase of the pulsation cycle.

In one herd (Chastain) the vacuum (milking) phase was shorter than the atmospheric pressure phase. This ratio was unsatisfactory because it slowed milking. For many herds, 50:50 (1:1), 60:40 (1½:1) and 66:34 (2:1) ratios are satisfactory. Limited observations indicate that a 3:1 ratio is unsatisfactory.

We feel that speeding up the milking process by widening the ratio will accomplish nothing unless the time the machines are on the cows is shortened accordingly. Failure to change machines faster will result in over-milking and more mastitis.

Udder Treatment:

Correcting mechanical problems greatly reduced, and in many cases almost eliminated, the need for udder treatments during lactation. It was found that cows with CMT reactions of "2" and "3" often harbor mastitis organisms in a quiescent state.

All dairymen in this study adopted a dry cow treatment program. In most herds only the "2" and "3" cows were treated. Some dairymen treated all quarters; others checked each quarter with the CMT paddle and reagent and treated only the infected quarters. In most cases, the dry cows were observed for any udder distentions during the first week or two after treatment. If any swelling was observed, cows were retreated.

In most cases, the selection of drugs for udder infusion was based on the recommendations of veterinarians. The recommended procedure is to run sensitivity tests on milk from approximately 10 percent of the consistently CMT-positive cows. From the results of these tests the veterinarian prescribes the drug or antibiotic of choice.

In Brief:

Beware high vacuum.
Avoid high milk lift.
Keep pulsators in good repair.
If not already using them, try narrow-bore liners.
Take advantage of natural milk letdown.
Don't over-milk.
Employ the California Mastitis Test monthly on each cow.
Treat CMT reacting cows in the dry state with the drug recommended by your veterinarian.

The dramatic success of the California Mastitis Testing program in Shasta County led to a statewide project, which the Agricultural Extension Service and the School of Veterinary Medicine have made available at the county level. The project is entitled "The Control of Bovine Mastitis." Today 26 counties in the state already have 164 demonstration herds functioning and comprising 20,219 cows and other counties are in the process of working out a modus operandi patterned after the Shasta County program.
That the foregoing figures are modest as to the number of California dairies involved on a CMT project and the number of dairy cattle being tested in the program—let me cite only four examples.

The cooperating engineer on the project reported to me November 25, 1959, that he has tested out the equipment on seven demonstration herds in Los Angeles County involving 1,868 cattle—in reality the Los Angeles Livestock Department—under the direction of Dr. R. J. Schroeder—reports 25 herds with 5,008 cattle involved.

Stanislaus County has had nine herds checked out involving 995 cattle. In a recent farm advisor monthly report from that county he now has 7,000 cows on the project.

In Fresno County the equipment on four demonstration herds has been checked out involving 755 cattle and as of last week, that farm advisor reported to me he is now assisting 35 dairymen.

Nor do I have the Tulare County figures recorded in my formal report where 125 shippers are being serviced by the Tular Dairymen Cooperative—a private enterprise.

So the figure of some 30,000 plus dairy cows is a modest report of the influence of the California Mastitis project which was initiated at the state level February 5, 1959:

ECONOMICS INVOLVED

In a study of the production of 3785 cows in Sacramento County the CMT negative cows produced more milk by:

- 2,178 pounds more than CMT traces
- 2,631 pounds more than CMT 1's
- 3,392 pounds more than CMT 2's
- 4,572 pounds more than CMT 3's

In one county, 66 percent of 475 cows in five herds picked at random were reactors of CMT two or more. One herd had 90 percent reactors, with a 30-pound milk production average, and another herd had 47 percent reactors, with a 52-pound milk production average.

It is not to be forgotten that Mastitis is a limiting factor in Sire selection.
CMT BULK SAMPLING ON THE FARM

Trace indicates that over 25 percent of the herd is affected.
1 indicates that as high as 50 percent of the herd is affected.
2 indicates that over 50 percent of the herd is affected.
3 indicates that over 75 percent of the herd is affected.

Any milk preservative other than refrigeration or 0.5 percent dry boric acid nullifies the CMT.

This constitutes a major bottleneck in the project during the summer months.

RECAPITULATION

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<th></th>
<th>Vacuum</th>
<th>Narrow Bore Lines</th>
<th>Inner Lining</th>
<th>Disinfect Between Cows</th>
<th>Weather Effect</th>
<th>Stimulation</th>
<th>Segregation</th>
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SUMMARY AND CONCLUSIONS OF THE SHASTA COUNTY CMT PROGRAM

Vacuum:

The 14 dairymen cooperating in this project were nearly unanimous in condemning high vacuum. Those who made the greatest progress attribute much of their improvement to lowering of vacuum.

Fluctuating Vacuum:

Almost every installation which initially employed a vacuum lift was altered to include a milk pump. The acute flare-ups of mastitis associated with fluctuating vacuum were more devastating than the CMT graph depicts.

The explanation for the difficulty with fluctuating vacuum seems to be as follows. Several inches of vacuum are expended in milking the cow and lifting the milk. The reduced vacuum at the teat end is in effect only as long as the udder secretes milk. When milk secretion stops, the vacuum level at the claw increases. To provide adequate vacuum for satisfactory milking and proper teat stimulation by the collapsing liner at the low ebb, the regulator
is often set to provide excessive vacuum at the other extreme. If not handled with extreme care, fluctuating vacuums are potentially dangerous.

**Pulsator Performance:**

Air leaks associated with worn piston-type pulsators were found to be a serious problem. Worn pulsators result in little or no rest phase in the pulsator cycle. This interferes with necessary blood circulation in the teat tissue. Serious damage can result if vacuum at the teat is not relieved.

Installations were found where master pulsators were called upon to perform beyond their capacities. This, too, results in little or no time interval for a rest phase in the pulsation cycle.

The Strain-Gage amplifier is an indispensable device for pinpointing pulsator problems. If trouble is prevalent, nothing about the pulsator can be assumed. One is completely in the dark until he knows the performance characteristics of all pulsators in use.

**Narrow-Bore Stretch Liners:**

Ten of the 14 dairymen changed to narrow-bore liners. One additional dairyman changed temporarily, but he returned to the large-bore liners. He found the Bou-Matic shell excessively long for the Surge pot with the plastic window in the telescopic shell expanded, which was a common and legitimate objection. There was general agreement that the narrow-bore stretch liners do a superior job of milking. All narrow-bore users reported faster and more complete milking.

**Liner Life:**

Since the narrow-bore stretch liner is usually manufactured with more natural rubber and is under tension, it has a much shorter life than the large-bore liner, which is made with a greater percentage of synthetic rubber and is under little or no tension. From 700 to 1,000 milkings seems to be a reasonable life expectancy for narrow-bore liners. The lower cost of the narrow-bore liner fully compensates for the shorter life.

**Disinfecting Teat Cups:**

Only five dairymen disinfected teat cups between cows. Adequate disinfecting implies that a disinfecting solution of proper strength is in contact with the internal liner surface for a sufficient length of time (at least 30 seconds in the case of chlorine solutions). There must be a bleed hole in the claw for air to escape. A “before and after” test was run in the Hall herd to test the effectiveness of disinfecting. The evidence here and in other herds indicates that disinfecting can be beneficial under certain conditions. Some herds do well without disinfecting.

**Weather:**

All dairymen recognized the correlation between wet weather and mastitis. However, only four rated weather as a major factor. These four dairymen were among those with the least mastitis problems.
This year's report of the Committee on Infectious Diseases of Cattle will be confined to one specific subject—morbidity and mortality reporting. Many of the major diseases of cattle are being reported on in other committee reports. These are cattle diseases which are obviously important problems to the livestock industry even though with the exception of brucellosis and tuberculosis we, for the most part, do not have valid statistics to confirm our general observations or no statistics at all.

The need for a comprehensive and meaningful animal disease morbidity and mortality reporting system needs no detailed justification. A valid statistical basis of animal disease incidence is a necessary prerequisite to stimulating and guiding both applied and basic veterinary research, to the planning and establishment of sound disease eradication programs, and to maintaining a successful defense against biological warfare or the accidental entrance of foreign diseases.

In evaluating the incidence of a particular disease and, more important, its economic importance, the livestock industry still gropes for these needed facts in a world of darkness. We have, it is true, made advances in recent years in attempting to gain such statistics and evaluations. Many states have established morbidity and mortality reporting systems although the degree of practitioner cooperation has been universally very poor. The Animal Diseases Eradication Division of the Agricultural Research Service has established an animal disease reporting system for collecting, coordinating and evaluating information received from the various states' morbidity and mortality reporting systems. Many special surveys are made by various research agencies, diagnostic laboratories and other agencies. Meat inspection services also provide statistics on disease diagnosis made by their examinations. There are, consequently, many sources of information regarding morbidity and mortality data. There is also an operating agency (the A.D.E.D.) to collect and evaluate such information.

This is a good beginning but much more needs to be done if morbidity and mortality reporting is going to achieve any degree of success. What is now needed to supplement and to activate the program is study and experimentation in two general fields: methods of collection of information and statistical methods of converting the information collected into valid estimates of disease incidence and their loss value in dollars and cents.
It is obvious that methods for collecting information on disease losses for a disease such as bovine mastitis is materially different than for bovine anthrax. Some disease losses may be more accurately evaluated by serological surveys; others by selecting sampling, and so forth. Methods of evaluating disease incidence involve many variables. Practitioner reports are subject to a representative sampling and accuracy in diagnoses problems. Serological or other surveys may vary considerably by the type of test used or even variables in a particular test. By varying the ambient temperature when rapid plate brucellosis agglutination tests are conducted, for example, the incidence of brucellosis may increase or decrease.

The establishment of such a reporting system will require the leadership of men with creative abilities to pioneer the field. It is the recommendation of this Committee that every effort be made to stimulate interest for research studies and experimentation in methods of collecting and evaluating morbidity and mortality statistics so that these statistics can be collected and converted into utilisable and meaningful information. It is also the recommendation of this Committee that funds be made available by the Agricultural Research Service of the United States Department of Agriculture for the employment of a staff composed of statisticians and veterinarians to develop a morbidity and mortality collection, reporting, and evaluation system which could be uniformly used by all states.
More than 40 years after the beginning of the campaign to eradicate tuberculosis from livestock, infected animals are still being found in every state in the nation. More important, in some areas during recent years the rate of infection has been on the increase.

During fiscal year 1959, a total of 8,187,161 cattle were tested for tuberculosis to find 18,914 reactors. This is the smallest number of cattle tested since 1945. The percentage of reactors found in 1959 was 0.23, which is the highest since 1946. And the number of accredited herds at the end of the year was 52,946, the lowest figure for herds in this status since 1924. Other data relating to the cooperative state-federal tuberculosis eradication program are contained in the statistical tables for this project. (A limited supply of these tables is available here, and additional copies may be obtained by contacting the ADE Division, Agricultural Research Service, Washington, D. C.)

Obviously, we are a long way from our goal of tuberculosis eradication. Yet there are encouraging signs. For example, livestock disease control officials and members of the livestock industry are showing far greater interest in this disease than at any time in recent years.

A. Supervision and Attention

During the past year there has been a growing trend toward providing more effective supervision and attention to the various phases of the tuberculosis program in all states. It is apparent that those responsible for the operation of the program in each state have given increased attention to the effectiveness of their over-all program, as well as attention to the proper direction and instruction of the individual operator. The importance of supervision must be continually stressed in all phases of the program if maximum results are to be obtained from testing efforts.

B. Conferences

When this Association met in Miami, Florida, in 1958, the Tuberculosis Committee endorsed plans for holding another conference on the subject of tuberculosis similar to that held in Michigan in June 1958. This recom-
mendation was followed, and a second conference of national scope was held at Kansas State University, Manhattan, Kansas, in August 1959.

This conference was attended by state and federal regulatory officials from 49 states, as well as representatives from Canada and England. In addition, the group also included representatives from veterinary colleges, public health departments and the field of human medicine. Included on the program were leading national authorities in research, education and regulatory services.

The discussions were highlighted by nine major themes involved in eradicating tuberculosis. These were:

1. A positive mental approach toward total eradication.
2. Getting maximum results from our tuberculin tests.
3. A better knowledge of the technical aspects of the disease.
4. Eliminate human exposure to cattle.
5. Broaden our outlook through epidemiology to effectively disclose sources of infection.
6. Remove causes of associated sensitivity.
7. Profit from world-wide knowledge.
8. Replace opinions with facts.
9. A comprehensive diagnosis.

Both the interest and enthusiasm felt by those attending the conference were emphasized in a concluding conference panel of state regulatory officials who expressed general agreement on the immediate need for a vigorous and continued attack on tuberculosis which would lead to early eradication of the disease.

To further highlight the problems in detecting and eliminating tuberculosis, another conference was held this past year for veterinarians in the State of Wisconsin. Here again it is felt that the benefits derived from this meeting have been well worth the effort in view of the greater attention being given the program in that area.

C. Special Field Projects

It has been recognized for some time that certain aspects of tuberculosis have warranted further study. Such current problems as the effect of avian tuberculosis, human tuberculosis, paratuberculosis, and possibly others, and their relation to present diagnostic procedures are being extensively reviewed. Special field investigations are being conducted in cooperation with several states to provide further information on these particular factors. Information gained from these special epidemiological projects may aid us in developing still more effective procedures for dealing with tuberculosis.

As has been pointed out, comprehensive field studies are being conducted in various states. For example, such a study is currently underway in Cache County, Utah. This work includes the application of tuberculin tests on cattle using mammalian and avian tuberculin, as well as tests on other species of livestock and poultry. Tissues from reacting animals are submitted to the ADE Diagnostic Laboratory at Ames, Iowa and the Veterinary Science Department of Utah State University for complete histological and bacteriological examination, including inoculation of laboratory animals.
Another special project is being conducted in Iowa, where efforts are being made to follow up on all cases of tuberculous swine found by federal meat inspectors at one slaughtering establishment.

This work includes studies at premises from which swine have been shipped and which have later disclosed lesions of tuberculosis on regular kill. Since these animals are purchased—and payment made by the packer—on the basis of actual carcass grade and yield, tattoo identification is necessary prior to slaughter in order that the owner will be properly identified. Thus, in those cases where infected animals are found at the time of slaughter, tracing of these animals to the farm of origin is relatively easy.

Testing of swine, cattle and poultry on the premises of origin, along with epidemiological studies at each farm, should provide further information on the extent and causes of tuberculosis in swine.

In Wisconsin, special projects are being conducted in two counties in cooperation with the Wisconsin Department of Agriculture and the Department of Veterinary Science, University of Wisconsin.

Studies are being made using a variety of tuberculins and Johnin to provide further evaluation of diagnostic agents. Tissues from reacting animals are processed at the University of Wisconsin Department of Veterinary Science. Culture work and animal inoculation is currently under way on these specimens, and epidemiological data gathered from these field studies are being analyzed.

In the State of Michigan, a field study is being developed cooperatively between the ADE Division, the Michigan Department of Agriculture, and Michigan State University. This program will be activated immediately following completion of plans for facilities, personnel, and other project details.

In addition to these special projects, comprehensive research studies on tuberculosis are being developed. The State of Wisconsin has made funds available for tuberculosis research at the University of Wisconsin. The Animal Disease and Parasite Research Division, Agricultural Research Service has provided funds for a research project at Michigan State University. It is hoped that these new projects in addition to those underway in connection with serological tests for tuberculosis and paratuberculosis and other work on paratuberculosis at the Animal Disease and Parasite Research Division at Auburn, Alabama, will provide information with which to more effectively combat these diseases. However, as pointed out in the 1958 report, it will be necessary to continue to utilize those techniques and procedures that have been so effective in the past while searching for new information needed to meet the challenge of changing conditions.

D. Post-Mortem Examination of Reactors

Progress has been made this past year in developing uniform procedures for conducting post-mortem examination of tuberculosis reactor animals in plants that do not have federal meat inspection. The Federal Meat Inspection Division has developed a training guide which gives specific instructions and information on the examination of reactors. Since 35 percent of all reactors
found in 1958 were handled in non-federally inspected plants, this provides a standard guide for uniform and thorough examination regardless of whether slaughter is under federal, state or municipal supervision.

The Federal Meat Inspection Division has designated 20 of its stations for training veterinarians in post-mortem examination procedures, and has been cooperating with the ADE Division and some state and municipal officials in making this specialized training available.

E. Epidemiological Investigations

The importance of epidemiology in dealing with tuberculosis has received increased attention during 1959, and it is especially important that additional stress be placed on this facet of the tuberculosis eradication program.

The various factors at each premise where reactors are found must be thoroughly investigated and effort directed toward disclosure and removal of the source of infection. In studying these factors, consideration must be given other species of animals, as well as man, as a potential source of infection. As a further aid in conducting these studies, an epidemiological guide has been prepared and is available for use in dealing with problem herd situations.

The movements of cattle associated with every infected herd must be successfully traced so that every possible foci of infection will be revealed. Considerable progress has been made both in determining the history of reactors and following up on exposed animals as disease control officials have become more familiar with the system of reporting the history and movement of animals to and from infected herds. However, steps must be taken to further improve this system through intelligent and diligent attention to each of these reports.

F. Tracing

The 1958 Committee on Tuberculosis of this Association stressed that greater attention be directed toward locating infected herds through tracing to the herds of origin animals that show lesions of tuberculosis on regular kill.

This question has been raised frequently in recent years: Should more reliance be placed on tracing these regular kill lesion cases and at the same time decrease the amount of testing presently being carried on?

In this connection, records that have been accumulated on herds with lesion reactors on only one test with no recorded history of prior infection and no subsequent infection as determined by one or more negative tests should be taken into consideration.

For example, 535 herds have been found in this group with only one reactor, 180 herds with two reactors, and 207 herds with three or more reactors, making a total of 922 known herds in this classification.

These 922 lesion reactor herds found as a result of over-all testing may be compared with 491 reactor herds found as a result of tracing lesion cases found on regular kill during the same period.
Failure to test all of the herds in either group would have resulted in additional foci of infection going undetected for a longer period during which an unknown amount of infection might have been spread to cause additional financial losses, danger to public health, and a further delay in attaining the goal of eradication.

There is room for considerable improvement in the efficiency of the present tracing program. And while it is anticipated that an increasing percentage of such cases will be successfully traced as better identification and more complete record systems are developed, it is presently felt that this procedure should continue as an adjunct to the regular testing program rather than as a substitute for these procedures as a means of locating tuberculous herds and animals.

It should be noted, that during the five years 1955-1959, a total of 72,005 tuberculosis reactors were slaughtered. Of these, 5,539, or a total of 7.6 percent, were found as a result of tracing to herds of origin animals that were reported with lesions on regular kill.

These reactors were found by testing 164,824 cattle, or only 0.38 percent of the total of 43,922,211 cattle tested during the five-year period. During this same period, 18,607 reactors with gross lesions were slaughtered. Approximately 19 percent (3,378) of the total lesion reactors were revealed as a result of these tracing investigations.

G. Federal and Non-Federal Plants

The location and proportionate number of slaughtering plants operating under federal inspection and plants not subject to federal inspection as of March 1, 1955, are of considerable interest. There were a total of 455 United States inspected plants, 952 non-federally inspected plants, and 1,810 smaller non-federally inspected plants. A large number of these small plants are operating in those states where there is a concentration of dairy and breeding animals.

H. Reactors Disclosed: Tracing Lesion Cases, Regular Kill

Reactors were disclosed in 25 states following reports of lesion cases of tuberculosis found on regular kill in plants operating under federal inspection during fiscal year 1959. During the same period, reactors were disclosed in seven states as a result of reports of tuberculous lesion cases in plants operating under inspection other than federal. Four of these seven states represent an important segment of the dairy producing area.

It is apparent that increased activity on the part of livestock sanitary officials and officials of local meat inspection agencies would provide valuable contributions to the eradication of tuberculosis and other communicable diseases affecting livestock. Special need exists in those areas where so many cattle from dairy herds are slaughtered in plants not subject to federal inspection.
I. Problem Herds

The attention of the ADE Division was recently drawn to a dairy herd with a long history of tuberculosis in one of the northeastern states. Authentic records show that tuberculosis has existed in that herd for at least 15 years. It is likely that the problem has existed for as long as 25 years.

Tuberculin test records on the herd for the period prior to 1947 have been destroyed. However, the owner stated that all but two animals reacted to the initial herd test, and indicated that these two animals were sent to slaughter with the reactors. It is reasonable to assume that this occurred in the early 1930's when the first area test was applied in the county.

Following is a summary of the interesting highlights taken from the official records for the herd:

The herd of 89 animals passed a negative test in December 1947. The record for this test includes the tag numbers of two animals that later showed lesions of tuberculosis on regular kill.

In November 1948, the carcass of a two and one-half year old heifer was condemned by a Federal Meat Inspector because of generalized tuberculosis. A herd test in less than a month was reported as negative. This was followed by another negative herd test a year later.

In June 1950, the same veterinary meat inspector reported lesions of tuberculosis in the carcass of a cow from the herd. On a test within 30 days approximately one-half of the herd was tested and found negative. The remaining animals were not brought in from a back pasture to be included in this test.

Six months later, in December 1950, six reactors were detected on the regular yearly test. All of these reactors were included in the prior annual test. Three were not listed on the record of the partial herd test in June, immediately after finding the lesion case on regular kill.

Between December 1950 and November 1959 a total of 80 reactors, which is approximately equal to the number of animals in the herd, have been found on 12 of 25 herd tests. The complete herd was not assembled during the summer months when nine of these 25 herd tests were made.

It is encouraging to note that the state livestock sanitary official and ADE Veterinarian in Charge are taking personal interest in this herd. They have recently visited the premises together to work out appropriate procedures for freeing this herd from tuberculosis as soon as possible.

There are undoubtedly various factors that collectively are responsible for the delay in eradicating tuberculosis from the herd. Almost anyone could pose as a “Monday morning quarterback” and cite what should have been done years ago. But are those responsible for tuberculosis eradication taking a careful look at all of the records to determine whether other similar herds exist? In addition, is proper attention being given to all infected herds to assure that complete investigations are made to prevent other herds from developing a parallel history of tuberculosis?

When infection is revealed, all available information concerning herd history and records of individual animals in the herd is of utmost importance.
During February 1959, a test was conducted in a herd of 28 cattle. One registered Holstein-Friesian cow reacted and showed well-marked lesions on autopsy. The reactor had been purchased in November 1958 from a local dealer. The dealer had previously sold this cow to a dairyman who returned it to the dealer the same month he purchased it—May 1959.

The cow had been in a herd from which a two-year old heifer was tanked for tuberculosis on regular kill in June 1958. A herd test conducted in September resulted in 35 percent of the animals reacting. The disease had become so extensive that it was decided to consign the entire herd to slaughter.

From the preceding examples it is obvious that when more comprehensive information is developed it becomes possible to locate new foci of infection.

If a complete epidemiological study of the herd had been made when infection was first found it would be reasonable to expect that the animal that passed through the herd and later reacted would have been located much sooner. This would have prevented the spread of considerable potential infection.

J. Status of Modified Accredited Areas

It is obvious that confidence in our modified accredited area status is generally lacking. Only eight states will accept without restrictions cattle that come from modified accredited areas in other states. Steps are being taken which we hope will assist in developing greater confidence in the modified accredited status.
Prompt reaccreditation of counties is an essential factor for the restoration of confidence in the health status of cattle moved interstate to farm areas. Definite progress has been made in this direction during the past year. The number of overdue counties has been reduced from 243 to 145. The number of counties overdue for one year or longer has been reduced from 63 to 32.

Fig. I shows the states and counties listed in these two categories.

The federal regulations for interstate shipments of cattle have recently been amended to provide for the removal of an overdue state or subdivision thereof from the modified accredited status, unless officials of such state or portion thereof have taken and are continuing to take satisfactory action to achieve reaccreditation. After July 1, 1960 additional time, not to exceed one year from the date reaccreditation was due, may be allowed in certain special cases for an area to obtain reaccreditation.

This amendment also requires that an area be reaccredited in accordance with provisions of the Uniform Methods and Rules for the establishment and maintenance of modified accredited areas. This is essentially as recommended by the Committee on Tuberculosis of this Association last year.

There is a wide variation in the percentage of cattle tested in the various states to qualify areas for modified accredited status. This spread extends from a low of three percent of cattle tested in some areas to a high of 100 percent in other areas.

The national average, based on requests for reaccreditation of counties submitted is 36.3 percent of the cattle population. The states have been grouped according to the relative percentage of cattle tested.

There are 2,781 requests for area reaccreditation that include statistical information. Of those requests 1,479, or 55 percent, indicate that no reactors or other evidence of tuberculosis were revealed.

Based on the same requests for reaccreditation, it is noted that 1,463 areas, or 53 percent, have been reaccredited for a period of 6 years.

K. Areas of Heavy Infection

Another significant factor is the percentage of total reactors removed in certain states during the period from 1917 through 1957. These are as follows: 69 percent in nine states; 24 percent in 13 states; and seven percent in 26 states.

The comparable figures for the same groups of states during fiscal year 1959 are: 83 percent in nine states (15,705 reactors); five percent in 13 states (1,029 reactors); and 12 percent in 26 states (2,180 reactors).

During fiscal years 1953 through 1959, 7,901 reactors were revealed as a result of tests after lesions of tuberculosis were found in animals subject to inspection on regular kill. Of those reactors 5,538 or 70 percent were revealed in the same nine states where the highest percentage of total reactors (1917-1957) were found.
Tuberculosis Eradication

**TUBERCULOSIS IN SWINE**

![Map showing the percentage of inspected swine found tubercular by state in Fiscal Year 1959.](image1)

% OF INSPECTED SWINE FOUND TUBERCULAR
- NONE
- 0.1-1.9
- 2.0-2.9
- 3.0-3.9
- 4.0-8.9

FISCAL YEAR 1959

SOURCE: FEDERAL MEAT INSPECTION REPORTS

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

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**TUBERCULOSIS IN FOWL**

![Map showing the percentage of inspected fowl found tubercular by state in January-September 1959.](image2)

% OF INSPECTED FOWL FOUND TUBERCULAR
- NONE
- 0.001-0.009
- 0.01-0.09
- 0.10-0.49
- 0.50-2.10

JAN.-SEPT. 1959

SOURCE: AGRICULTURAL MARKETING SERVICE

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
From data supplied by the Meat Inspection Division, Agricultural Research Service, we find that 63,870,479 swine were inspected in fiscal year 1959 to find 1,785,902 retained because of tuberculosis. This is 2.78 percent of those examined. Swine carcasses condemned and passed for cooking amounted to 9,263. The percent of swine carcasses inspected in the various states and found to have tuberculosis have been placed in five groups as illustrated in the map, Figure II. A similar breakdown of data furnished by the Inspection Branch, Poultry Division, Agricultural Marketing Service, for tuberculosis in fowl is shown on the map, Figure III.

In considering these illustrations it should be pointed out that both swine and poultry may be shipped varying distances to slaughter and many lots may be slaughtered outside the areas in which the particular lot was grown.

From the preceding report on the current status of the tuberculosis eradication program it is obvious that, while progress is being made, much remains to be done before this disease can be completely eliminated from the livestock population of this country.

With the increased interest, support, and spirit of cooperation shown by livestock sanitarians, producers, the public, and others who have such a vital stake in wiping out this disease we can look to eradication as a reality and not merely a goal.
TUBERCULOSIS REACCREDITATION AND ERADICATION
IN A WESTERN RANGE AREA

J. W. Safford, B.S., D.V.M., Montana State Veterinarian

Montana, a western range and semirange state, has a 46-year history of concentrated effort to eradicate bovine tuberculosis. We are sure this history is similar to that of other western range states. We believe a brief review of that history is necessary to better evaluate statements that will be made later.

The present population of Montana is 600,000 people, 98,000 dairy cattle and about 2,000,000 beef cattle. The area of the state is about 50 acres to each cow. Average annual precipitation varies in different parts of the state from about seven inches to 24 inches. At one time the only cattle that were housed during the winter were dairy cattle. In recent years a good share of the dairy cattle are no longer kept in large, damp barns all winter because of the adoption and use of milking parlors. The vast majority of cattle in Montana have never been in a barn. Quite a number have never been in a barnyard or small corral. Ranch management and environmental conditions in the range and semirange areas have never been conducive to the transmission of tuberculosis.

Prior to 1900 most of the ranches in Montana had become established and were stocked primarily from the trail herds originating from the Southwestern and Western United States. By 1900 the mining camps had become towns and places to raise families. Immediately following 1900, homesteads were being claimed which resulted in more families settling the land. This created a demand for milk and dairy products. As a result, many dairy cattle were imported from the Great Lakes region. A subcutaneous tuberculosis test was made on a number of these dairy cows in 1911 and 10.6 percent of them were tuberculosis reactors. During 1913 and 1914 this test was applied to 12,000 dairy cattle. The reactor rate was about four percent.

The intradermal test was adopted by the Montana Livestock Sanitary Board in 1914 as an official tuberculosis test. Since that time proportionately large numbers of cattle have been tested for tuberculosis each year up to the present time.

In 1916 all imported dairy cattle were required to be tested for tuberculosis on arrival. That year 1,389 cattle from the Great Lakes region were tested for tuberculosis and 10.3 percent were reactors. The following examples were not unusual. One shipment of 44 dairy cows from Wisconsin had 42 reactors. A shipment of 50 head from New York, that year, had 44 reactors. From 1913 to 1919 a total of 85,689 dairy cattle were tested and 3,654 tuberculosis reactors were found—a 4.2 percent reactor rate.

For the first time in the early 1920's, over 8,000 range cattle were tested and not one tuberculosis reactor was found. Through the entire history of

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Tuberculosis reaccreditation and eradication

Tuberculosis testing in Montana, a tuberculosis reactor in a range or semirange cow has been a rare finding.

Montana started the area plan to eradicate bovine tuberculosis in 1925. By 1935, the entire state was declared a modified accredited area. Over 1,900,000 cattle had been tested in Montana up to and including 1935. A total of 10,818 cattle were positive to the test—an overall tuberculosis infection rate of 0.56 percent.

Montana livestockmen are very proud of this accomplishment. They have been repaid many times over for their investment in time and money required to stop the economic and public health waste of bovine tuberculosis.

The 46-year tuberculosis test record is summarized in the following table:

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<th>Year</th>
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<th>Reactors Number</th>
<th>Reactors Percent</th>
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<td>85,689</td>
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<td>201,971</td>
<td>27</td>
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<td>34,460</td>
<td>3</td>
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</table>

All 56 Montana counties have been reaccredited every three years since the entire state became accredited in 1935. No tuberculosis reactors have ever been found in six Montana counties, either on original accreditation or on the subsequent reaccreditations. No tuberculosis reactors have been found in 27 of Montana’s 56 counties since the state was accredited in 1935—each county has been reaccredited eight times. No tuberculosis reactors have been found in 47 of Montana’s 56 counties in the last three accreditation tests or since 1949. A total of 333,981 cattle have been tested in 47 counties since the last reactor was found in those counties.

We must ask, and rightfully so, when is the end in sight? How much reaccreditation does it take to indicate that tuberculosis has been eradicated? How can we justify the expense of continued testing of cattle in counties that have not had a tuberculosis reactor in their history, or in 24 years, or in nine years, or in eight to three accreditation periods? How can we justify the expense of testing 8,000 to 11,000 cattle to find one atypical reaction that turns out to be a skin lesion case or is found to have no gross lesions, when retests of the herd of origin of such a reactor never reveal more reactors?

Let us examine very closely what we are actually doing in an attempt to ferret out that last tuberculosis-infected cow. We are attempting to locate 0.008 percent of infection by means of the reaccreditation plan. Present methods and rules state that range and semirange counties may be reaccredited for six years if 10 percent of the cattle have been tested and the reactor rate is less than 0.2 percent of the cattle tested. The average county in Montana has about 35,000 cattle. That means 3,500 cattle must be tested each six years. It will take 60 years to test all the cattle equal to the present population of the county. Under this reaccreditation plan of retesting the
It would take 60 years to try and locate 0.008 percent reactors. The cattle population is not static. During this 60-year period the cattle in that county will have produced an estimated 1,260,000 head of cattle. Yet, in that 60-year period only 35,000 head of cattle will have been tested to keep the county reaccredited. This means that only three percent of the total 60-year cattle population will have been tested for tuberculosis. It is obvious that the possibility of the reaccreditation plan finding a possible focus of infection in extremely low-tuberculosis-incidence areas is so remote that the present reaccreditation plan for a western range county is of no value and a needless expense.

Perhaps, and I only say perhaps, because I am not familiar with the tuberculosis-eradication problems in other regions of the United States, the dependence upon this reaccreditation test plan is responsible for the increase of bovine tuberculosis in the higher tuberculosis-incidence areas of the United States.

We believe that most tuberculosis-infected herds in the western range and semirange areas, the last two decades, have been located by finding cattle with tuberculosis lesions on the slaughter floor. This is not surprising because the numerical possibility of detecting sources of tuberculosis on the slaughter floor are so very much greater than finding them on the area reaccreditation test plan.

There must be a method developed and used to pinpoint sources of tuberculosis to avoid costs of large numbers of unnecessary reaccreditation tests in the range and semirange areas. A number of western states have called attention to a sound and practical procedure for ultimate tuberculosis eradication. As far back as 1932 the late Dr. W. J. Butler, Montana State Veterinarian from 1913 to 1948, advocated that post-mortem reports be utilized to locate sources of infection and negative tuberculosis post-mortem findings be utilized to reaccredit counties. Again in 1948 he recommended the use of post-mortem findings to eradicate tuberculosis. “We feel it is the one way by which tuberculosis can be ultimately and completely eradicated and the only way by which it can be satisfactorily and economically eliminated from the range states.”

In Montana 94 percent of the off-farm slaughtered animals are slaughtered under federal or state meat inspection. Through the brand inspection service of the state, 95 percent of the cattle slaughtered in Montana can be traced back to the ranch of origin. Out-of-state buyers of Montana slaughter cattle can trace back their purchases for the purpose of determining future purchases in regard to dressing percentages and parts condemnations. We feel confident that with a little additional effort, a greater and satisfactory percentage of “lesion cases” can be traced back to the ranches of origin. The back-tag program now being developed will be of valuable assistance in identification of cattle showing lesions at slaughter.

It is imperative that federal and state meat inspection services be more fully utilized in not only tuberculosis control and eradication programs but all animal disease control and eradication projects. All suspected tuber-
culosis lesions from cattle should be taken for laboratory confirmation and typing.

All herds suspected of being herds of origin of cattle showing any tuberculosis lesions on slaughter should be immediately tested and retested until there is no doubt that the disease has been eliminated. Should tuberculosis lesions repeatedly appear in cattle from a certain area and it appears that all foci of infection are not being eliminated by herd tests alone, then all cattle in that area should be tested for tuberculosis. All reactor herds should be rigidly quarantined until retests of the herd leave no doubt that tuberculosis has been eradicated.

Historically the tuberculosis problem in Montana was one of the dairy herds. Dairy herds will continue to be periodically retested to comply with the United States Public Health Service and Livestock Sanitary Board grade A milk requirements. This required testing of dairy herds along with the maximum utilization of post-mortem lesion cases found at slaughter will, we are confident, lead to complete eradication and/or assurance that complete eradication is maintained in a range and semirange area. This procedure will eliminate the costly unnecessary and no longer useful reaccreditation field testing of range and semirange cattle where tuberculosis is and has been nonexistent for many years.

We believe it is most important that the bovine tuberculosis eradication problem must be considered and evaluated on a regional basis. There is a great difference in climatic, environmental and animal husbandry conditions and practices from one region to another. Careful consideration must be given to those regions where the incidence of bovine tuberculosis is comparatively high and where it is low and nonexistent. Then, the most adaptable and practical plan for that area must be applied.

In the range and semirange regions the area accreditation and reaccreditation plan has very successfully accomplished a great deal toward tuberculosis eradication. It has established an extremely low incidence of tuberculosis. So low, in fact, that from cost standpoint and from the numerical improbability that it will ever find that last tuberculosis reactor, there is no longer justification for the area reaccreditation test plan in the range and semirange areas. We must stop thinking in modified-accredited tuberculosis-free area terms and start thinking and planning in tuberculosis-free area terms. After 46 years, bovine tuberculosis has either been eradicated from a county or it has not. We should discontinue qualifying the program with the term "modified." We recommend that the present area plan of reaccrediting modified-accredited areas be discontinued in the range and semirange areas. Counties should no longer be designated as modified-accredited free areas. They should be designated as tuberculosis-free or not tuberculosis-free.

In lieu of the modified-accredited area plan, we recommend a program for range and semirange areas based on the following general principles:

1. Counties or areas be declared tuberculosis-free if during the past few years there has been a complete absence of reactors to the tuberculosis test
and complete absence of tuberculosis lesions in slaughtered cattle that have originated from that county or area.

(2) Counties or areas should be maintained as tuberculosis-free as long as no tuberculosis lesions are reported in cattle which have been slaughtered and have originated from the county or area, and dairy herds fail to show reactors to tuberculosis tests that are required to be tested to meet United States Public Health Service Grade A dairy requirements.

The determination that cattle are free of tuberculosis lesions at slaughter would not be made from negative post-mortem reports, but from the absence of post-mortem reports showing tuberculosis lesions. We cannot expect state and federal meat inspection divisions to supply post-mortem reports on all cattle not showing lesions. We cannot expect state offices to provide time and space to file such unnecessary reports. This could involve reports on about one million head of cattle a year from Montana alone. Definitely, every lesion of tuberculosis found on post-mortem examination should be immediately reported to the state of origin.

(3) A county or an area would be suspended from the tuberculosis-free status if a single tuberculosis lesion was found in cattle originating from that county or area or a tuberculosis reactor was found in that county or area. The suspension would remain in effect until the herd of origin and all exposed cattle have been tested and declared free from tuberculosis according to present procedures of eradicating tuberculosis from a herd.

(4) Should repeated reactors appear in the county or area and should cattle be repeatedly found with tuberculosis lesions originating from a county or an area, the tuberculosis-free status of the county or area should be revoked. The entire county or area should be placed under a federal and state quarantine. All cattle in the county or area should be tested for tuberculosis. The infected herds should be retested, until there is no doubt that tuberculosis has been completely eradicated. When this is achieved the county of area would be restored to the tuberculosis-free status.

(5) All cattle shipped, other than for immediate slaughter, from counties or areas that have had the tuberculosis-free status revoked, would be required to be negative to a tuberculosis test prior to shipment from that county or area.

(6) Herds of origin of cattle showing tuberculosis lesions and herds of origin in which tuberculosis reactors are found should be rigidly quarantined. No cattle should be permitted to move from this herd, except for immediate slaughter, until the herd has been declared free from tuberculosis according to present herd test procedures.

We believe such an approach is now in order. A vigorous testing program in herds where indicated by slaughterhouse post-mortem reports will: (1) avoid unnecessary waste of manpower and funds in counties where bovine tuberculosis is nonexistent as now required by reaccreditation tests; (2) direct the tuberculosis tests where needed, and (3) assure that the range and semirange areas will remain free from tuberculosis.
PRELIMINARY REPORT OF INVESTIGATIONS OF TUBERCULIN SENSITIVITY IN WISCONSIN CATTLE *

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In 1940 the Committee on Tuberculosis of this Association called attention to the problem of no visible lesion reactors and pointed to the necessity of research in this area. This has been re-emphasized in almost every one of the Committee's reports in the intervening 19 years. Reactions to tuberculin in the absence of demonstrable tuberculosis have become a world-wide problem with increased tuberculin testing. Coincident with emphasis on the application of rigorous criteria of interpretation of the intradermal test during recent years, the problem has become acute in the north central states.

**TABLE 1**

Statistics from Wisconsin Tuberculosis Control Program—1956 to 1959

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Tested</th>
<th>Number Reactors</th>
<th>Percentage</th>
<th>Number Tested</th>
<th>Number Reactors</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>37,727</td>
<td>281</td>
<td>0.7</td>
<td>816,869</td>
<td>421</td>
<td>0.05</td>
</tr>
<tr>
<td>to 1957</td>
<td>No gross lesions</td>
<td>229</td>
<td>81.4</td>
<td>347</td>
<td>82.4</td>
<td></td>
</tr>
<tr>
<td>1957</td>
<td>27,556</td>
<td>638</td>
<td>2.3</td>
<td>815,946</td>
<td>1,469</td>
<td>0.18</td>
</tr>
<tr>
<td>to 1958</td>
<td>No gross lesions</td>
<td>509</td>
<td>79.8</td>
<td>1,280</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td>1958</td>
<td>23,271</td>
<td>1,322</td>
<td>5.7</td>
<td>583,381</td>
<td>2,995</td>
<td>0.51</td>
</tr>
<tr>
<td>to 1959</td>
<td>No gross lesions</td>
<td>1,149</td>
<td>86.9</td>
<td>2,526</td>
<td>84.3</td>
<td></td>
</tr>
</tbody>
</table>

In Wisconsin there has been a marked increase in number of herds containing reactors and numbers of reactors disclosed annually since July 1956 (Table 1). There has also been a marked increase in the number of NVL reactors. Although the absolute number of cases with lesions disclosed in

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§ Animal Health Division, Wisconsin State Department of Agriculture.

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creased during this period, analysis of post-mortem inspection reports reveals that the increase was confined largely to skin lesions and lesions in areas other than the thoracic cavity.

Several hypotheses have been considered in accounting for this rise in the incidence of tuberculin sensitivity. The first is that the test results are an index of a real increase in the incidence of bovine tuberculosis. This appeared to be unlikely for several reasons. First, the areas involved have been tested extensively and regularly over a period of many years with a regular decline in incidence of reactors prior to 1957. Second, over 90 percent of Wisconsin cattle slaughtered are subjected to federal inspection, and the incidence of tuberculous lesions found on regular kill has declined during this period of time. Third, the average incidence of less than two reactors per herd is not typical of spreading infection. Fourth, the high incidence of NVL reactors is not typical of spreading infection.

An alternative hypothesis is that a major share of the reactions are the result of sensitization to mycobacteria other than Mycobacterium bovis. There is abundant evidence, which has been reviewed recently by Paterson (5), that cattle may develop sensitivity to mammalian tuberculin by infection with M. paratuberculosis, M. avium, the organisms which cause skin tuberculosis, and possibly other mycobacteria. It is unlikely that a high incidence of heterologous sensitization is a new phenomenon. It may have been detected during the past decade but ignored on the basis of the diagnostic judgment of the veterinarians conducting the tests. It is also possible that there has been a real increase in reactions disclosed because of a general change to the use of 0.1 ml of tuberculin rather than amounts ranging from 0.03 ml to 0.08 ml, because of greater potency of newer lots of tuberculin in detecting heterologous sensitivity, or a combined effect of these. Comparative tests have been used with considerable success in Britain (6) and Germany (3, 8) to detect heterologous sensitivity.

In this paper preliminary data from one of several surveys designed to determine the factors involved in Wisconsin are reported. Because of the preliminary nature of the report, a complete survey of the pertinent literature is not included.

MATERIALS AND METHODS

Herds in six townships of Sauk County scheduled for initial re-accreditation tests under the area program were selected for the survey. All tests were administered by three teams of regularly employed state-federal veterinarians. Each animal received 0.1 ml of tuberculin from current production lots of so-called “contract” tuberculin intradermally in the left caudal fold. The classification and disposition of the animal as a reactor, deviator or negative cow was determined on the basis of the size of the reaction 72 hours after injection, using the standards suggested by the Animal Disease Eradication Division, i.e., reactors, P₁ or X₂. In addition to this standard test, a comparative test was made in the right caudal fold. Approximately 1/3 of the animals received 0.1 ml of tuberculin which had been manufactured by the Agricultural Research Service; 1/3 received 0.1 ml of avian tuberculin;
received 0.05 ml of the "contract" tuberculin. The magnitude of response to both injections was recorded at 48 and 72 hours.

One hundred seventy-two animals branded as reactors on the official test were subjected to additional comparative tests within 48 hours after the caudal test was read. Eleven different intradermal injections were made in randomly selected spots on both sides of the neck. The materials injected were: "contract" tuberculin, 0.1 ml and 0.05 ml; Agricultural Research Service tuberculin, 0.1 ml and 0.05 ml; avian tuberculin, 0.1 ml and 0.05 ml; Johnin, 0.1 ml and 0.05 ml; Weybridge PPD avian tuberculin, 0.1 ml; Weybridge PPD mammalian tuberculin, 0.1 ml; 0.85 percent saline solution containing 0.5 percent phenol and 0.5 percent glycerol, 0.1 ml. Measurements, to the nearest 0.5 mm, of the skin thickness immediately after intradermal injection and after 48 hours were made with a Hauptner skin thickness gauge, recorded, and the increase in skin thickness calculated. The locations of the injections were coded and the personnel conducting the tests did not know which preparation had been inoculated into the sites until the study was completed.

After completion of the comparative tuberculin tests, the animals were consigned to slaughter at federally-inspected packing plants in the usual manner. The comparative cervical tests were also applied on 117 animals in 17 herds in which no reactors had been disclosed on the standard caudal fold test.

At the packing plants, tissues from each animal were placed in pint ice cream containers for transportation to the laboratory. The materials obtained included: any grossly visible lesion, retropharyngeal, bronchial, mediastinal, portal, and mesenteric lymph nodes, ileocecal valve and adjacent intestine. At the laboratory these tissues were kept frozen at \(-20^\circ\) C until they could be processed.

The specimens were prepared for culture first by searing the outside surface, then cutting them into small pieces with sterile scissors. The tissue fragments were transferred to 300 ml Virtis homogenizing flasks, suspended in four percent NaOH and homogenized in an aerosol free assembly until a uniform suspension was obtained. The contents of each flask were transferred to a 150 ml plastic centrifuge bottle and the material sedimented by centrifugation at 3,000 RPM for 15 minutes, after which the supernate was decanted and the sediment neutralized with HCl. Smears for staining and microscopic examinations were prepared directly from the sediment and tubes of culture media were inoculated. In most cases four tubes of Lowenstein-Jensen medium containing glycerol were inoculated. In some cases an additional group of tubes of Lowenstein-Jensen medium without glycerol were used. These were incubated at 37\(^\circ\) C and examined weekly for two months. Bacterial colonies appearing during the incubation period were checked for the presence of acid fast cells by microscopic examination of Ziehl-Neelsen stained smears.

The tissue concentrates were stored at \(-20^\circ\) C in the plastic centrifuge bottles for future use in animal inoculation studies.

The ileocecal valves were prepared for culture in the same fashion, but the tissue concentrates were inoculated onto four slants of Smith's medium for *Mycobacterium paratuberculosis* (7).
The results of the official caudal fold tests are summarized in Table 2. Only 25.2 percent of the herds and 87.4 percent of the animals were classified as completely negative. Fifty percent of the herds (331) contained reactors but did have animals which were classified as deviators. Deviators were also found in 131 of the herds with reactors. A total of 1,764 or 10.8 percent of the cattle tested manifested the deviator response to tuberculin. Of the 16,348 cattle tested, 284 or 1.8 percent developed reactions classified as $P_1$, $X_2$ or greater. These were found in 160 or 24.4 percent of the herds.

If this hypersensitivity were an accurate index of infection with $M. bovis$, it would represent an incidence of epizootic proportions. It is difficult to reconcile such an interpretation with the fact that the average reactor herd contained only 1.8 reactors. The results of the post-mortem examinations of the 284 reactors, summarized in Table 3, are remarkable for the absence of lesions typical of infection with $M. bovis$. All but three of the 16 “slight lesions” in areas other than the thoracic cavity were solitary lesions in mesenteric lymph nodes.

### TABLE 2

Results of Official Caudal Fold Tests in Sauk County Project

<table>
<thead>
<tr>
<th>Herds</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactors*</td>
<td>160</td>
</tr>
<tr>
<td>Deviators†</td>
<td>331†</td>
</tr>
<tr>
<td>Negative</td>
<td>166</td>
</tr>
<tr>
<td>Totals</td>
<td>657</td>
</tr>
</tbody>
</table>

* Size of reaction recorded $P_1$, $X_2$ or greater.
† Any palpable deviation less than $P_1$ or $X_2$.
‡ Herds containing deviators but no reactors.

### RESULTS AND DISCUSSION

At the present time primary cultures from lymph nodes of all reactors have been completed. Acid fast bacteria have been isolated from the tissues of 14 animals from 12 different herds. Speciation has not been completed and, at this time it can be said only that one culture is dysgonic and resembles $M. bovis$, two resemble $M. avium$. The others are eugonic, pigmented, and
grow as well in the presence of glycerol as in its absence. It should also be pointed out that the tissues have not yet been inoculated into laboratory animals. It is to be expected that additional strains of acid fast bacteria may be isolated by this means.

The comparative tests in the caudal folds were designed to furnish answers to three questions: (1) With the standard field procedure of classifying response, is there a difference in potency between current lots of "contract" tuberculin and the tuberculosis previously manufactured by the Agricultural Research Service? (2) Is there a difference in the magnitude of responses elicited by 0.1 ml of "contract" tuberculin and 0.05 ml of "contract" tuberculin? (3) Is there a difference in the magnitude of responses elicited by 0.1 ml of avian and "contract" tuberculins?

The veterinarians who conducted the tests reported orally that they felt there was close agreement between the standard and comparative tests. This impression was not borne out by analysis of their recorded data, summarized in Table 4. It may be seen that of 136 reactors 14 (10.3 percent) developed only the deviator response to Agricultural Research Service tuberculin. A dose-response relationship is clearly seen in the fact that of 81 animals which developed a $P_1$, $X_2$ or larger reaction to 0.1 ml of contract tuberculin, 20 (24.6 percent) developed smaller reactions to 0.05 ml of the same tuberculin. It is clear from these data that the relative potency of a given lot of tuberculin, the quantity used, or both, could have considerable effect on the numbers of reactors detected in an area in which standard criteria of classification of reactions are employed. It is not possible to make an exact estimate of these effects because of the relatively small numbers of animals in each subgroup and the probable variation of interpretation among individual operators.

These effects could be measured with more precision in the 172 reactors given comparative tests in the cervical skin as shown in Table 5. It may be seen that most animals reacted to each of the tuberculins and that 13 percent had responses of two mm or greater to the control preparations.

Of the 117 cows from herds completely negative on the caudal fold test, 61 developed reactions of two mm or more on the cervical test with "contract" tuberculin. The percentage of animals reacting to the other allergens was proportionately less and the mean reaction sizes were less than those in the animals classified as reactors on the caudal test.

<table>
<thead>
<tr>
<th>Classification</th>
<th>0.1 ml ARS Tuberc.</th>
<th>0.1 ml Avian Tuberc.</th>
<th>0.05 ml &quot;contract&quot;</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactors</td>
<td>122</td>
<td>39</td>
<td>61</td>
<td>222</td>
</tr>
<tr>
<td>Deviators</td>
<td>14</td>
<td>11</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>136</td>
<td>67</td>
<td>81</td>
<td>284</td>
</tr>
</tbody>
</table>
### TABLE 5

*Increase in Skin Thickness on Comparative Cervical Tests of Reactors*

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Number Cattle</th>
<th>Dose ml</th>
<th>Mean Response (mm)</th>
<th>2 mm</th>
<th>3 mm</th>
<th>4 mm</th>
<th>5 mm</th>
<th>6 mm</th>
<th>7 mm</th>
<th>8 mm</th>
<th>9 mm</th>
<th>10 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Contract&quot;*</td>
<td>171</td>
<td>0.1</td>
<td>5.5</td>
<td>166(97)</td>
<td>149(87)</td>
<td>124(73)</td>
<td>95(55)</td>
<td>63(37)</td>
<td>42(24)</td>
<td>32(19)</td>
<td>25(15)</td>
<td>19(11)</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.05</td>
<td>4.3</td>
<td>155(91)</td>
<td>131(77)</td>
<td>91(53)</td>
<td>56(33)</td>
<td>39(23)</td>
<td>27(16)</td>
<td>21(12)</td>
<td>18(11)</td>
<td>13(8)</td>
</tr>
<tr>
<td>A. R. S.†</td>
<td>172</td>
<td>0.1</td>
<td>4.7</td>
<td>153(89)</td>
<td>127(74)</td>
<td>99(57)</td>
<td>63(37)</td>
<td>45(26)</td>
<td>30(17)</td>
<td>26(15)</td>
<td>22(13)</td>
<td>16(9)</td>
</tr>
<tr>
<td></td>
<td>169</td>
<td>0.05</td>
<td>3.6</td>
<td>144(85)</td>
<td>111(66)</td>
<td>81(48)</td>
<td>49(29)</td>
<td>31(18)</td>
<td>23(14)</td>
<td>21(12)</td>
<td>18(11)</td>
<td>12(7)</td>
</tr>
<tr>
<td>Avian</td>
<td>172</td>
<td>0.1</td>
<td>3.5</td>
<td>152(88)</td>
<td>103(60)</td>
<td>66(38)</td>
<td>40(23)</td>
<td>26(15)</td>
<td>17(10)</td>
<td>10(6)</td>
<td>5(3)</td>
<td>4(2)</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>0.05</td>
<td>2.9</td>
<td>140(82)</td>
<td>94(55)</td>
<td>56(33)</td>
<td>27(16)</td>
<td>16(9)</td>
<td>12(7)</td>
<td>7(4)</td>
<td>4(2)</td>
<td>3(1)</td>
</tr>
<tr>
<td>Johnin</td>
<td>168</td>
<td>0.1</td>
<td>4.5</td>
<td>159(95)</td>
<td>128(76)</td>
<td>96(57)</td>
<td>69(41)</td>
<td>40(24)</td>
<td>31(18)</td>
<td>24(14)</td>
<td>15(9)</td>
<td>9(5)</td>
</tr>
<tr>
<td></td>
<td>169</td>
<td>0.05</td>
<td>3.5</td>
<td>146(86)</td>
<td>98(57)</td>
<td>72(43)</td>
<td>48(28)</td>
<td>27(16)</td>
<td>15(9)</td>
<td>11(7)</td>
<td>6(4)</td>
<td>5(3)</td>
</tr>
<tr>
<td>PPD Mammalian</td>
<td>172</td>
<td>0.1</td>
<td>3.9</td>
<td>157(91)</td>
<td>121(70)</td>
<td>78(45)</td>
<td>52(30)</td>
<td>33(19)</td>
<td>19(11)</td>
<td>15(9)</td>
<td>12(7)</td>
<td>10(6)</td>
</tr>
<tr>
<td>PPD Avian</td>
<td>172</td>
<td>0.1</td>
<td>3.9</td>
<td>144(84)</td>
<td>103(60)</td>
<td>63(37)</td>
<td>45(26)</td>
<td>34(20)</td>
<td>26(15)</td>
<td>20(12)</td>
<td>17(10)</td>
<td>15(9)</td>
</tr>
<tr>
<td>Control</td>
<td>171</td>
<td>0.1</td>
<td>0.4</td>
<td>23(13)</td>
<td>6(3)</td>
<td>4(2)</td>
<td>4(2)</td>
<td>1(0.6)</td>
<td>1(0.6)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

* Current production lots of tuberculin.
† Lot manufactured by Agricultural Research Service.
TUBERCULIN SENSITIVITY IN WISCONSIN CATTLE

These results were to be expected in view of the known greater reactivity of the cervical skin (1), but they confirm the existence of wide spread low level sensitivity in many cattle.

A dose-response relationship was marked with each tuberculin. This is evident in the mean responses and even more striking when the numbers of animals with reactions of a given size are tabulated as they are in Table 5. For example, of 171 animals tested with 0.1 ml of "contract" tuberculin, 166 (97 percent) had reactions of two mm or greater; 149 (87 percent), three mm or greater; 124 (73 percent), four mm or greater. When the dose of the same tuberculin was reduced to 0.05 ml, the proportions changed to: 155 (91 percent), two mm or greater; 131 (77 percent), three mm or greater; 91 (53 percent), four mm or greater.

Differences in potency are also apparent in the mean responses and in the proportions of animals with reactions of different sizes. The "contract" tuberculin employed elicited larger reactions and more animals developed reactions of a given size to it than to the Agricultural Research Service tuberculin, the mammalian PPD, or any of the non-mammalian tuberculins. The lower potency of the ARS tuberculin may be attributable to loss of potency because of its age (2).

Although the occurrence of sensitization to Johnin and avian tuberculins was anticipated (5), the incidence and the degree of this sensitization were greater than had been expected. A considerable proportion of animals which developed any arbitrarily selected size of reaction to "contract" tuberculin also developed reactions of the same size to avian tuberculin or Johnin. For example, 55 percent of the cows had an increase in skin thickness of five mm or more in response to the "contract" tuberculin; 23 percent had reactions of the same size to avian tuberculin; 41 percent had reactions of this size to Johnin. If specificity of response is interpreted as a greater response to allergen from the sensitizing organism than to heterologous allergens, a considerable amount of sensitization to organisms other than M. bovis may be assumed to have occurred in these cattle. One measure of specificity would be to require that the response to one allergen exceed that to the others by some arbitrarily selected amount, say one mm. The specificity index in this series using this criterion is shown in Table 6. This type of analysis cannot be taken as an exact index of the nature of the sensitization as information is not available on the relative homologous potencies of the various allergens used (5).

|TABLE 6|

Distribution of Specificity of Hypersensitivity

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Contract&quot; tuberculin</td>
<td>43 percent</td>
</tr>
<tr>
<td>Avian tuberculin</td>
<td>6 percent</td>
</tr>
<tr>
<td>Johnin</td>
<td>18 percent</td>
</tr>
<tr>
<td>No specificity*</td>
<td>33 percent</td>
</tr>
<tr>
<td>PPD mammalian</td>
<td>50 percent</td>
</tr>
<tr>
<td>PPD avian</td>
<td>28 percent</td>
</tr>
<tr>
<td>No specificity</td>
<td>22 percent</td>
</tr>
</tbody>
</table>

* No specificity = equal response to two or more allergens.
A discrimination test which required the response to "contract" tuberculin to exceed that to avian tuberculin and Johnin by only one mm would have reduced the condemnations in this series by 57 percent. If the most stringent of the interpretations of the British discrimination test (4) were applied to the results with the PPD tuberculins, only 10 animals would have been condemned. Among the 14 animals from which acid fast bacteria have been isolated, there were none which would have been condemned by this discrimination test.

Although final evaluation of these data will depend upon completion of the bacteriological studies, they seem to us to require consideration of a number of questions in respect to our tuberculosis eradication program.

1. In view of the widespread occurrence of low level sensitivity to tuberculin, are the potency and quantity of tuberculin used excessive for populations with a low incidence of infection?

2. In view of the relatively low degree of specificity of the responses to mammalian tuberculin, should a discrimination test be employed? Work to be reported elsewhere with another large group of cattle has shown a very large number of these reactions to be transitory.

3. Perhaps the most important question concerns the source of the apparently hetero-specific sensitization. It is possible that significant information may be developed from work with the mycobacteria which have been isolated from the 14 cows in this series as well as a larger number of strains recovered from animals in another survey.

REFERENCES


REPORT OF THE COMMITTEE ON TUBERCULOSIS


Your Committee on Tuberculosis again presents its annual report. For many years this Committee has directed its attention to activities in connection with eradication of bovine tuberculosis. It is increasingly evident that tuberculosis of all species of livestock must be included in our eradication efforts. Thus, epidemiological surveys of livestock must be extended to include studies on the relationship of tuberculosis in all animals and humans. These surveys must also include special studies relative to para-tuberculosis.

Complete epidemiology will direct us in determining the course we shall take in regard to continued research on tuberculosis. We are glad to note that research projects are now underway in Wisconsin and Michigan; the first such project in individual states in many years. While extensive research is greatly needed and may disclose information of benefit, there is no reason at the present time to discount the tuberculin test as an effective means of locating tuberculous animals.

All agencies involved in tuberculosis eradication activity must accept their responsibility in carrying out the program requirements as set forth in the Uniform Methods and Rules. These methods and rules must be strengthened from time to time as gains are made.

Your Committee believes that standards for a tuberculosis-free area should be developed and incorporated in the Uniform Methods and Rules. In view of the task of developing acceptable standards for such areas we urgently recommend this matter be considered by next year’s committee.

As a step towards tuberculosis-free areas consideration must be given to eliminating reaccreditation by testing a relatively small percentage of the cattle in the area. State agencies must prepare themselves for more comprehensive testing for reaccreditation.

Your Committee recommends The Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, work with the tuberculosis committee of this Association and such other agencies as they may choose in developing and publishing a “Fact Sheet” on tuberculosis for the benefit of livestock owners. Such “Fact Sheet” shall include the relationship between bovine, avian and human tuberculosis and para-tuberculosis.

Your Committee desires to call the attention of the members of the Association to the progress that is being made in redeveloping interest in tuberculosis eradication. We particularly direct your attention to the excel-
lent conferences developed by the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, that have been so beneficial in restimulating interest and enthusiasm in this project. Your Committee recommends that additional conferences be held on national and/or regional bases in order that those engaged in eradicating tuberculosis may be better fitted to carry on their work.

Your Committee further recommends the Uniform Methods and Rules be amended to read as follows:

**UNIFORM METHODS AND RULES FOR THE ESTABLISHMENT AND MAINTENANCE OF TUBERCULOSIS-FREE ACCREDITED HERDS OF CATTLE AND MODIFIED ACCREDITED AREAS**

**PART I**

**Individual Accredited Herd Plan**

1. (a) A tuberculosis-free accredited herd is one in which no reactors have been found on at least two consecutive annual tuberculin tests. Herds in which reactors occur shall be quarantined and must successfully pass a tuberculin test after a period of at least 60 days before the herd may be released from quarantine. If there is indication of advanced tuberculosis in one or more reactors, a second test at least 60 days from the prior negative test, and such additional tests as the cooperating state and federal officials deem necessary shall be applied before releasing the herd from quarantine. All herds in which reactors occurred shall be retested in approximately 12 months but not more than 15 months following the first negative test after disclosure of reactors, at which time the herd may be accredited or re-accredited if it otherwise qualifies. These tests are to be followed by at least two annual herd tests, which shall be applied by a veterinarian employed in a full-time capacity by the state or the federal Animal Disease Eradication Division or by an accredited veterinarian.

   (b) When an accredited herd test or other tests are applied by an accredited veterinarian a report of such tests shall be submitted in accordance with the regulations of the cooperating state and federal authorities. These officials reserve the right to supervise any tests conducted by an accredited veterinarian.

   (c) When suspects to the tuberculin tests are disclosed in herds not containing reactors, such animals shall be quarantined to the premises where disclosed (accredited herd status to be suspended) and no movement of animals from the herd will be permitted except under permit issued by the cooperating state authorities until the status of the herd is determined by a retest of the suspect not less than 60 days subsequent to the original test. A complete herd test shall be conducted when the suspects are retested or within a period of six months.

2. (a) The official tuberculin test shall be the intradermic test. The intradermic injection shall be a measured amount of tuberculin, 0.1 cc. for routine testing 0.2 cc. for retesting herds when advanced tuberculosis has been
disclosed. The intradermic injection of tuberculin in the cervical area shall
be made only in herds where advanced tuberculosis has been disclosed and
then only by a full-time employed state or federal veterinarian.

3. The entire herd, or any cattle in the herd, shall be tuberculin tested or
retested at such times as are deemed advisable by the cooperating state and
federal authorities.

4. No animal that has been designated a reactor at any time shall be
retested.

5. (a) Reactors to the tuberculin test shall be removed from the farm in
accordance with state and federal laws and regulations. After their removal
the infected premises shall be thoroughly cleaned and disinfected with a
disinfectant approved by the United States Animal Disease Eradication Di-
vision, in a manner satisfactory to the cooperating state and federal author-
ities.

(b) A complete epidemiological survey shall be made on all herds in which
reactors are disclosed, by appropriate state or federal personnel.

6. Herd additions must originate directly from tuberculosis-free accredited
herds or herds in a modified accredited area that were tested and found
negative within a 12 month period immediately prior to being added to the
herd. Other cattle to be added to an accredited herd, shall originate directly
from modified accredited areas, pass a negative test not more than 30 days
prior to entry and be segregated from the remainder of the herd until re-
tested and found negative at least 60 days after entering the premises where
the accredited herd is maintained.

7. To qualify for accredited status all animals must be bona fide members
of the herd. A certificate issued jointly by the local state and federal officials
shall be in effect. The accredited herd status may be valid for not more than
one year (365 days) from the date of the qualifying herd test. To qualify
for reaccreditation the herd must pass a satisfactory test within a period of
15 months from the last previous accreditation test.

8. Owners of accredited herds shall be required to maintain such environ-
mental conditions as are consistent with the generally accepted standards of
good sanitation and herd management. The use of milk or other dairy
products for feeding is prohibited unless such products are from a known
safe supply or have been pasteurized or sterilized. Identity shall be provided
by ear-tag or other satisfactory means for all animals and complete records
of all additions to the herd must be kept. Only properly clean and disinfected
vehicles may be used for transporting cattle into accredited herds.

9. Failure on the part of an owner to comply with these methods and
rules shall constitute sufficient cause for the revocation of the accredited
herd certificate.

PART II

Modified Accredited Area Plan

10. The provisions of the individual accredited herd plan that relate to
testing removal of reactors, cleaning, disinfecting, sanitation and epidemiol-
ogy shall apply to the modified accredited area plan. All suspects must be
retested or slaughtered and subjected to a postmortem examination com-
parable to that required for reactors before calculating the percentage of
infection for the county.

11. Modified accredited areas may be reaccredited for a period of six
years if, as a result of a retest of all cattle in the area, the degree of infection
based on the last test of each herd discloses not more than 0.2 percent of the
cattle population of the area.

12. Modified accredited areas that disclose on the last test of all cattle not
more than 0.2 percent infection may be reaccredited for a period of six
years if a retest of 10 or more per cent of the cattle in said area discloses
a degree of infection not exceeding 0.2 percent. In calculating the degree of
infection all post-mortem meat inspection reports of tuberculosis and other-
wise disclosed cases of tuberculosis accumulated in said area since the last ac-
creditation test must be included. All herds containing reactors with advanced
tuberculosis disclosed in the county within the past 12 years shall be included.

13. Modified accredited areas that disclose on the last test of all cattle
more than 0.2 percent infection may be reaccredited for a period of three
years if a retest of 20 or more percent of the cattle in said area discloses a
degree of infection not exceeding 0.5 percent. In calculating the degree of
infection all post-mortem meat inspection reports of tuberculosis and other-
wise disclosed cases of tuberculosis accumulated in said area since the last
test for accreditation must be included. All herds containing reactors with
advanced tuberculosis disclosed in the county within the past 12 years shall
be included.

14. A county or area may be reaccredited in the range or semi-range
region upon compliance with paragraph (a), (b), or (c) of this section
provided all infected herds disclosed in the county during the preceding 12
years are tested during the period of reaccreditation.

(a) A modified accredited area in the range and semi-range region may be
reaccredited for a period of three years if the total number of reactors is not
more than 0.5 percent of all cattle tested in the area provided:

(1) That all commercial dairy herds and all registered pure-bred
herds are tested.
(2) That 20 percent of all farm herds are tested (a new group of
farm herds shall be tested until all in the area have been included).
(3) That 5 percent of the semi-range breeding females are tested.
(4) That such other cattle as may be considered necessary by the
state and federal cooperating official are tested.

(b) A modified accredited area in the range and semi-range region may
be reaccredited for a period of three years provided:

(1) That all commercial dairy herds and all registered pure-bred
herds are tested.
(2) That 20 percent of all farm herds are tested (a new group of
herds shall be tested until all in the area have been included).
(3) That reports are produced showing that during each year at least five percent of the range and semi-range breeding cows in the area as determined by statistics of the A.M.S. or a total of 15 percent during a three-year period have been subjected to a post mortem examination at an approved establishment.

(4) That all cattle in herds of origin or cattle associated with those showing evidence of tuberculosis at time of slaughter are immediately tuberculin tested in accordance with the provisions of the accredited herd plan (paragraph 1, Part I).

(5) That the percentage of infection disclosed as a result of such tests as conducted under the provisions of this paragraph does not exceed 0.5 percent of the area cattle population (excluding untested quarantined feeder cattle); the number of reactors used in computing the percentage of infection to be the number accumulated over the three-year period.

(c) A modified accredited area in the range and semi-range region may be reaccredited for a period of six years if the total number of reactors is not more than 0.2 percent of all the cattle tested in the area provided:

(1) That all commercial dairy herds and all nonrange farm herds and all registered pure-bred herds are tested.

(2) That all herds found to be infected and all herds adjacent to infected herds are placed under quarantine and tested as required in paragraph 1 (a), Part I.

(3) That reports are produced showing that during each year at least five percent of the range and semi-range breeding cows in the area as determined by statistics of the A.M.S. or a total of 30 percent during the six year period have been subjected to a post mortem examination at an approved establishment.

(4) That all herds found to be infected on post mortem examination shall be placed under quarantine and tested as required in paragraph 1(a), Part I, and all herds adjacent to infected herds shall be also tested as required in Paragraph 1(a), Part I.

(5) That such other cattle as may be considered necessary by the state and federal cooperating officials are tested.

If a retest of an area as provided for in this paragraph discloses more than 0.5 percent infection, accreditation shall be suspended until the above provisions have been met during a subsequent 18-month period except that the number of animals required to qualify under 14 (b) (3) shall be at least 7.5 percent of the range and semi-range breeding cows in the area.
Definitions

1. A "range animal" is interpreted to mean one maintained on natural forage and/or browse during the entire calendar year.

2. A "semi-range animal" is interpreted to mean one maintained on natural forage and/or browse during the entire calendar year with occasional supplemental feeding.

3. A "commercial dairy herd" is a herd made up of cattle of the recognized dairy breeds from which milk is produced and sold as such for human consumption.

4. A "registered pure-bred herd" is one that has a minimum of 10 registered pure-bred cattle during all parts of the year and from which registered animals may be sold as such for breeding purposes.

5. A "farm herd" in the range and semi-range region is made up of cattle kept to supply milk and dairy products for home use, and those from which limited sales of milk or cream are made.

6. A "Testing on a continuing basis" where all the cattle in certain designated and clearly established portions of a county are tested during a specified period (year) so that all herds within the county will have been tested within a six year period.

7. "Advanced tuberculosis" refers to carcasses of animals in which a lesion of tuberculosis is found as a result of post-mortem examination.

8. "Annual tests" for purposes of accreditation means tests made in not less than 11 nor more than 15 months.

9. "Quarantined feeder cattle"—cattle of the beef type maintained under strict quarantine for a limited period and marketed in a manner that will assure immediate slaughter.
RECENT STUDIES ON THE BIOLOGICAL AND PHYSICAL CHARACTERISTICS OF THE AVIAN LEUKOSIS VIRUSES

B. R. Burmester, Ph.D., D.V.M.*

Ellermann (1) was the first to recognize the three general types of leukosis in the chicken as erythoid, myeloid and lymphoid in reference to neoplasia of the three main blood cell types. Whereas, the first two mentioned are generally leukemic, the lymphoid type is usually aleukemic and has generally been known as visceral lymphomatosis (2) or lymphocytoma (3). Recently, in a workshop conference held at the United States Regional Poultry Research Laboratory, it was agreed that the designation “visceral lymphomatosis” is to be regarded as a broad term indicating neoplasia of all of the lymphocytic series, which, in individual cases, shows varied degrees of malignancy, as indicated by the relative immaturity of the cells and/or relative proportion of immature cells present (4). This is in conformity with the classification made by Jungherr (2) and is consistently used at the United States Regional Poultry Research Laboratory (5, 6, 7, 9, 8).

This definition of visceral lymphomatosis, however, is not in agreement with that of a group of English workers (9, 10, 11, 12) who use the term in a much more restricted sense. Campbell (10) has described tumors of the viscera, especially the gonad, which closely resemble the lesions of the nerve and iris in cases of neural and ocular lymphomatosis. Such tumors are thought to be the result of an inflammatory response rather than being neoplastic; and therefore are considered by them to be a visceral form of fowl paralysis (neural lymphomatosis) or they may be referred to simply as visceral lymphomatosis. Lymphoid tumors showing anaplasia are classified by Campbell as lymphoid leucosis.

It would appear that the classification of visceral tumors as suggested by Campbell can be done only after careful histologic studies. The etiologic relation between the various forms of lymphomatosis and between these and lymphoid leukemia still remains an open question. The primary evidence that can be cited is based on epizootiologic observations. However, experimental evidence continues to accumulate indicating that the etiology of the visceral lymphomatosis, classified by Campbell (10) as lymphoid leukemia, is different from that of fowl paralysis (neural lymphomatosis), and a visceral form of the latter is a possibility that should be given consideration.

A classification and terminology of disease which is based on etiology is much more appropriate and useful than one based on pathology; however, until the etiology of fowl paralysis in all of its “forms” has been determined it would seem inappropriate and cause much additional confusion to again change the nomenclature.

The viruses causing the three distinct leukotic diseases: myelo (granulo) blastosis, erythroblastosis and visceral lymphomatosis (lymphoid leukemia) are present in the plasma at relatively high concentrations. This was the first source of virus for study with the electron microscope. Examination of films from plasma or ultracentrifuged preparations have revealed spheroidal particles that vary widely in size but average about 120 μ in diameter (13, 14, 15). When ultra thin sections were made of particles from plasma of chickens with erythro or myeloblastosis they measured about 80 μ in their long axis and usually appeared to be short oval in shape (16). The particles contained electron dense central bodies, presumably, primarily ribonucleic acid, which were 30-40 μ in diameter and limiting membranes which often are seen double. This type of ultra structure, size and shape, appears to be common to all chicken tumor viruses thus far studied.

Myeloblastosis: Studies of ultra thin sections of bone marrow, spleen, and liver of chickens with myeloblastosis and of myeloblasts in tissue culture reveal that typical particles are present in the cytoplasm (17, 18). The number of virus particles seen in these cells was remarkably few in view of the high rate at which virus was liberated by cells in tissue culture and the large amount of extra cellular virus in the culture fluid and in plasma of diseased birds (19). This probably indicates that virus released from the cell must closely follow its formation.

By culturing myeloblasts in 50 percent chicken serum Bonar (19) was able to obtain high yields of virus and to observe a series of changes in the cytoplasm of the myeloblast which strongly implicated the mitochondria and the “gray bodies” or viroplasts as major sites of virus synthesis. Related vesicles and vacuoles appeared to be involved in the maturation and liberation of the particles. The process of virus synthesis continues over long periods with no apparent ill effects to the myeloblast. During cell division infectious material is distributed between daughter cells. Infection of additional mitochondrial centers has been suggested to occur through the medium of ribonucleic acid derived from centers of virus synthesis.

The studies of Haddad et al. (20) have provided convincing evidence for the site of virus synthesis and the basis for its enzyme activity. They made ingenious correlative studies using the light microscope (phase contrast and light and dark field illumination) of living cells supravitally stained and later fixed and treated to demonstrate specific structures, and the electron microscope to study ultra thin sections of the same material.

Structures designated as viroplasts in electron micrographs have been identified as such under the light microscope (20). Specific reagents demonstrated a strong adenosine triphosphatase activity which was limited to the viroplast and it was clear that the mitochondrion was negative in this respect. Since ample evidence has been presented which shows that this enzyme is an integral part of the myeloblastosis virus, the foregoing observation provided further evidence that virus synthesis related to adenosine triphosphatase occurs in the viroplast. A part of the enzyme activity of the viroplast was
associated with the gray, amorphous ground substance in which virus particles are embedded; thus the gray substance may, in part, be used in the synthesis of the virus.

The developing granules of the normal myelocyte strongly dephosphorylated adenosine-triphosphate and there is some indication that such granules originate in the mitochondrion. It has been suggested (20) that the infectious process involves the mitochondrion at the precursor stage in the development of the granule; such a structure could well contain the enzymes required for the synthesis of a virus having adenosine triphosphatase activity (20).

Erythroblastosis: The virus particles as seen in the spleen and bone marrow of chickens with erythroblastosis are similar in size, shape and structure to those in tissues of myeloblastosis (19, 21, 22). The particles were found either in intercellular spaces, in the cytoplasm near the periphery of the erythroblast or in vacuoles or inclusion-like bodies of erythroblasts, macrophages and reticulum cells. The vacuole containing particles of the erythroblast and possibly the reticulum cell were considered to be of mitochondrial origin. Presence of individual particles free in the cytoplasm may be due to rupture of vacuoles or may represent early stages of cell degradation. Expelling virus particles through the cell membrane by a process of “budding” has been described (19, 21).

Iwakata and Amano (23) have reported that only immature virus particles are found in the erythroblast. These particles are without central dense bodies, are larger (92 mu) than mature particles and are spherical instead of oval in shape. Their findings suggest that the immature virus of the erythroblast is released within cytoplasmic protruberances as in the process of budding and these cytoplasmic enclosed particles are engulfed by phagocytes, where not only maturation takes place, but also replication of particles. This peculiar symbiotic interrelationship has been termed “fosterage proliferation” by Iwakata and Amano (23).

These findings are at variance with those of Benedetti and Bernhard (21), Dmochowski et al. (22, 24) and Bonar et al. (19) who find mature particles in erythroblasts. The presence of mature particles in macrophages is considered incidental to virus formation and the result of the natural activities of macrophages to engulf debris.

Visceral lymphomatosis: Only preliminary studies have been made concerning the virus-cell interrelationship in visceral lymphomatosis. Dmochowski et al. (25) found that the size, shape and structure of virus particles as well as their location and distribution in the tumor cells of the spleen and liver of chickens with visceral lymphomatosis were similar to that found in chickens with erythroblastosis. The inclusion-like bodies (viroplasts of Bonar et al.) (19) containing numerous virus particles have been found also to contain what appeared to be broken cristae and to be surrounded in places by a double membrane. These structures indicate that such inclusion bodies are of mitochondrial origin similar to that found in erythro and myeloblastosis.

Normal chicken tissue: Particles indistinguishable from those described as virus have been found in very low numbers in tissues of a low percentage of normal chickens and chick embryos (26, 27). It has been suggested that
such particles are either nonspecific saprophytic agents with an ultra structure similar to oncogenic virus or that they represent a latent infection of one of the tumor viruses. It is well known that the visceral lymphomatosis virus infection is widespread among all stocks of chickens, is transmitted through the egg and causes inapparent infection in the embryo and chicken resulting in a contamination of all tissue cultures containing chick embryo or chicken materials.

STUDIES IN TISSUE CULTURE

Myeloblastosis: Studies at Duke University (28, 29) have contributed much to an understanding of virus growth in naturally infected cells. Myeloblasts obtained from the blood of chickens with myeloblastosis have been in continuous culture for periods up to six months in Gey's salt solution with added chicken serum. Rates of virus output by these cells was measured by: (1) direct enumeration in the electron microscope, (2) by the activity of the tissue culture fluids to dephosphorylate adenosine triphosphate, and (3) by assay in susceptible chickens. Virus output was greatly influenced by the amount and source of chicken serum. Twenty percent or more serum with added glucose and folic acid gave optimum growth.

The rate of virus liberation was greatest during the two to 12 day period, during which the average of a series was 31 particles per cell per hour. The rate then decreased but continued for long periods indicating a process of progressive and continuous virus synthesis. The cell population remained in balance by multiplication at a slow rate which replaced degenerated cells. This slow death rate suggests a very harmonious parasitism between the virus and the infected myeloblasts.

Myeloblasts taken from the bone marrow of normal chickens have been maintained in tissue culture infected with BAI strain A virus and has resulted after an interval of 12 to 20 days in neoplastic transformation, as manifested by an increase in multiplication of the cells with associated liberation of myeloblastosis virus. The morphology of such altered cells and their rate of virus output were similar to myeloblasts obtained from diseased chickens.

Lymphoid tumors: Davis and Gustafson (30) grew primary explants of lymphomatous tissue and organs of chickens with intramuscular transplants of strain RPL12 tumors (31). Outgrowths of the various elements including lymphoid tumor cells occurred within 24-48 hours. After two to 13 days, supernatants were transferred to primary chicken embryo spleen culture and passages made at intervals of three to seven days. Supernatant fluids were tested by inoculating chickens intramuscularly. Results presented show that the lymphoid tumor was cultured in vitro through 10 passages with outgrowths of neoplastic cells. However, interpretations with regard to the propagation and activity of a virus are questionable. Only two of the supernatant fluids tested were passed through porcelain filters. One of these caused neoplasms in 45 percent of the chickens; the tumors were located at the site of inoculation. No evidence of the retention of test bacteria by the filter was given and cell-free inoculums of strain RPL12, though known to contain virus(es) causing visceral lymphomatosis, erythroblastosis and osteopetrosis, have never
caused any sort of tumor at the site of inoculation, whereas, tumor-cell suspensions, even when containing only a few cells per inoculation almost always cause the growth of a lymphoid tumor at the point of inoculation (31, 32). Although virus may have been present in the cultures proof that tumors in the chickens were due to virus activity is lacking.

Cytopathogenic agent of strain RPL12: Fontes et al. (33) and Sharpless et al. (34) obtained from the chicken propagated strain RPL12 a cytopathogenic virus which after the first few passages could be grown to titers of $10^8$ TCID$_{50}$. Both the epithelial cells and fibroblasts of chick embryo liver or of whole chick embryos were visibly affected in two to four days incubation.

Plaque formation was observed by Levine and Sharpless (35) and Stoker (36) in monolayers of chick embryo liver and in agar suspension of trypsinned chick embryo cells. In the former preparation, microscopic plaques could be counted in two to three days, and with the latter method, grossly visible plaques could be seen after five to seven days incubation when stained with neutral red. This virus can thus be assayed easily and accurately by the plaque count method.

Defendi and Sharpless (37) have described in detail the morphological and cytochemical changes observed in tissue culture and in chicken embryos infected with the tissue culture virus. In the tissue culture system large intranuclear inclusions rich in desoxyribonucleic acid and eventually leading to cell destruction were observed. The following series of changes were observed in 12-day embryos inoculated intravenously. In 0-48 hours, intranuclear inclusions formed in the hepatic cells, similar to those observed in tissue culture. In 48-72 hours, large basophilic cells proliferated in the mesenchyma of the liver and other organs. Later, large areas of necrosis appeared in the liver. The proliferative process was considered similar to the initial phase of experimental lymphomatosis in the adult and was prevented by the tissue culture virus antisera.

The tissue culture virus has been visualized in electron micrographs by Davis and Sharpless (38). Sprayed preparations have consistently shown spherical particles about 90 mu in diameter. Particle counts and infectivity titers were found to be consistently correlated giving an average number of particles per tissue culture infectious dose of five for one tissue culture strain and 35 for a second. Further identification of the particles as the infectious agent was obtained by observing, under the electron microscope, the agglutination of virus particles by specific antiserum. Ultra-thin sections of infected cells in tissue culture were studied by Davis et al. (39) who found that the virus particles were 85 mu in diameter with a central core of 44 mu, and all particles appeared to be confined to the nucleus. The latter is in agreement with that of Defendi and Sharpless (37), who found large intranuclear inclusions rich in desoxyribonucleic acid.

The early results gave presumptive evidence that the tissue culture cytopathogenic virus was identical with or closely related to, the virus causing visceral lymphomatosis in chickens. More recent data place a question on such a relationship. Sharpless et al. (34) reported visceral lymphomatosis in 15 to 56 percent of chickens inoculated with virus that had gone through
eight or more serial passages. They also reported neutralization with homologous antiserum with titer up to 1:2048 and with immune serums to strain RPL12 lymphomatosis, strain R erythroblastosis and strain A myeloblastosis. Antisera to other viral diseases of chickens were negative.

Fontes et al. (33) obtained a high incidence of lymphomatosis in the chickens inoculated with the second and third passage fluids but a low incidence with fluids of the tenth passage. Neutralization results were similar to those reported by Sharpless et al. (34). Defendi and Sharpless (37) reported 22 of 24 chickens developed visceral lymphomatosis after inoculation with tissue culture virus of the fourteenth serial passage.

Recent studies at the United States Regional Poultry Research Laboratory (unpublished) reveal that the tissue culture virus antiserum, which, in high dilution will neutralize 1000TCID₅₀ doses, has no detectable neutralizing effect on the strain RPL12 chicken propagated virus causing visceral lymphomatosis and erythroblastosis. A serum prepared by Doctor Sharpless gave similar results. In view of the foregoing, and the repeated observation that antiserum against the strain RPL12 chicken virus will neutralize at high dilutions the tissue culture virus, it would appear that the latter antigen was present in both the chicken strain and the tissue culture strain, but the lymphomatosis antigen was present only in the chicken propagated strain.

Additional infectivity tests have revealed that supernatant fluids of the eighth, thirteenth and eighteenth tissue culture passages did not cause lymphomatosis in chickens and five tissue culture materials supplied by Doctor Sharpless of several passages and/or strains were also negative in the causation of visceral lymphomatosis. These results are at variance with the early data and place a definite question on the possible relationship between the tissue culture virus and that causing visceral lymphomatosis in chickens. Further evidence that the tissue culture cytopathogenic virus is not related to a leukosis virus is found in the reports of Defendi and Sharpless (37) and Davis et al. (38) who found viral inclusion bodies and actual virus particles in the nucleus. This is contrary to all the detailed studies of several investigators who have found that the virus particles in all of the avian neoplasms studied are confined to the cytoplasm of the cell or may be intercellular.

These data could be explained by the existence of a virus which grows well in chickens without causing overt disease and is propagated serially in the tissues of the chicken used for the passage of strain RPL12. In tissue culture it grows rapidly resulting in high titers, and causing cytopathology, whereas, the visceral lymphomatosis virus grows only slowly, if at all, in tissue culture and eventually is lost with continued serial passage.

THE INTERRELATIONSHIP BETWEEN VISCERAL LYMPHOMATOSIS AND OTHER TRANSMISSIBLE NEOPLASMS

Research at the United States Regional Poultry Research Laboratory has shown that under experimental conditions, there is an intimate relation between the occurrence of visceral lymphomatosis and other neoplasms, especially, erythroblastosis (8, 40). All sources of virus thus far examined
which cause visceral lymphomatosis have also caused other neoplasms, thus it can be said that no “pure” strains of visceral lymphomatosis have as yet been isolated. This situation has been one of the greatest obstacles in the progress of research on avian neoplasms because convincing evidence as to whether each neoplasm is caused by a separate specific virus, or that a single multipotent virus is responsible for all neoplasms, or that single, double and triple potent viruses may be involved, has not as yet been obtained.

When conditions conducive to the full expression of the potentialities of the virus or viruses of a leukosis strain are not provided, misleading interpretations may result. Examples of this are the two strains extensively studied by Dr. J. W. Beard and co-workers (41, 42). Using inbred line 15, White Leghorns of the Regional Laboratory, and observing them for periods of about four weeks, they obtained only myeloblastosis after inoculation with the BAI strain A and only erythroblastosis after inoculation with the Engelbreth-Holm strain R; this led to the conclusion that these strains were pure.

Conditions necessary for the full expression of oncogenic viruses include the following: The host chickens should be not only highly susceptible genetically, but also without maternal antibodies, and should be exposed at an age of optimum susceptibility; the dose of virus must be such as to result in some birds surviving the disease(s) which occurs early, yet be sufficiently large to give some expectancy of positive transmission; and the experimental period must be of a duration to permit the development of all possible neoplasms.

Burmester et al. (40) reported on the neoplasms obtained with the plasma of the erythroblastosis strain R and the myeloblastosis strain A in chickens of inbred line 151 when they were held for a period of 270 days. It was found that, of the chickens that were inoculated with strain R and survived the early erythroblastosis, a high percentage developed visceral lymphomatosis. Strain A inoculated birds that survived the early myeloblastosis, developed not only visceral lymphomatosis but also renal adenocarcinomas and osteopetrosis. All sorts of combinations of the last three neoplasms were observed. Furthermore, the viruses of both strains were transmitted by bird to bird contact causing all the neoplasms found in the inoculated chickens except myeloblastosis. Infectious levels of virus were found in oral washings and extracts of droppings from chickens that had been inoculated and of others in direct contact with chickens inoculated with either strain R or strain A. These sources of virus caused the same spectrum of neoplasia as the plasma of diseased chickens.

Although the virus particles of these two strains are indistinguishable morphologically and are serologically closely related, there are marked differences between them which cannot be ignored (19, 42). These are based on, (1) adenosine triphosphatase activity, (2) Forssman antigen, (3) precipitation reaction, (4) virulence or pathogenicity and (5) shift in sensitivity related to age. In view of these differences, how can one explain the causation of the same disease, visceral lymphomatosis by the two strains, especially at quite high dilutions? If a visceral lymphomatosis was present in
both strains as a contaminant, its concentration undoubtedly would be low and may not be manifested at the dilutions of plasma used.

Virus obtained directly from a flock with concurrent mortality from visceral lymphomatosis, or that obtained from lymphoid tumors after only a few serial transfers in susceptible chickens, have invariably caused erythroblastosis and visceral lymphomatosis and often also, osteopetrosis.

Extracts of embryo liver of 28 different normal hens have caused leukosis in 12 to 63 percent of those inoculated with an over-all distribution of 3.1 percent erythroblastosis and 25.5 percent visceral lymphomatosis. Oral washings and fecal extracts of nine different hens have caused an average of 2.7 percent erythroblastosis and 25.2 percent visceral lymphomatosis. Extracts of incubator debris from the hatching of naturally infected hens have caused 5.5 percent erythroblastosis and 52.1 percent visceral lymphomatosis.

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Of 19 transplantable lymphoid tumors propagated from field cases of visceral lymphomatosis, seven were found to contain a filtrable infectious agent(s) causing both visceral lymphomatosis and erythroblastosis. Five of the seven also caused osteopetrosis (8). The relative proportion of the various neoplasms occurring in any inoculation depends not only on the viral attributes of the inoculum but also on such factors as the route of inoculation, the dose of inoculums, and the age and the genetic constitution of the chickens.

Studies (8) with strain RPL12 have shown that the large dose of virus caused primarily erythroblastosis with death occurring within 100 days, while moderate and small doses caused visceral lymphomatosis after 100 days. The dose-erythroblastosis relationship was linear at the high dosage range, whereas, the dose-visceral lymphomatosis mortality relation was linear only at the low dosage range. Thus the types of leukosis obtained with strain RPL12 were to a large extent, determined by the dose. A similar and somewhat less predictable relation was obtained with strain R as given above.

Sensitivity of chickens to develop primarily erythroblastosis decreases with various routes of inoculation in the following order: intramedullary, intravenous, intraperitoneal, intracerebral, intramuscular, and subcutaneous. The two natural routes, oral and nasal, caused much lower responses than any of the above parental routes and most, but not all, of the mortality was due to visceral lymphomatosis. There was obtained a great variation between different routes in the proportion of erythroblastosis to visceral lymphomatosis mortality but none of the routes were effective in causing only one or the other of the two diseases. In view of the marked influence of dosage it is probable that the variations in response with the different routes were primarily, if not wholly, due to variations in effective doses; i.e., the amount of virus in contact with the sensitive cells.

In experiments involving direct contact of non-inoculated chickens with others inoculated with a high dose of strain RPL12 it was found that whereas the latter, as expected, died at a high rate with erythroblastosis within 100 days, the contact birds developed primarily visceral lymphomatosis after 120 days of age. This qualitative difference in the primary disease obtained may well be due simply to the much smaller effective virus dose received by the contact chickens than was received by the inoculated chickens.
The change in sensitivity of chickens at different ages was found to vary with the route of exposure (44). When large or small doses were given intravenously resulting in primarily erythroblastosis or primarily visceral lymphomatosis respectively, the responses varied little if at all with age during the first two weeks of life, then decreased at a uniform rate until at least 12 weeks of age, at which time the LD$_{50}$ was two to three logs greater than during the first two week period. A much more rapid decrease in sensitivity was noted when inoculations were made by the intraperitoneal route; the LD$_{50}$ increased three logs by the time the chickens were three weeks of age. An even more rapid decrease was noted when the chickens were infected by the oral and nasal routes; however, despite this rapid loss in sensitivity chickens nine weeks of age developed up to 17 percent visceral lymphomatosis.

The development of osteopetrosis after intravenous inoculation of strain RPL12 appears to be much more sensitive to the age influence than is erythroblastosis or visceral lymphomatosis; thus the percentage osteopetrosis in chickens inoculated at three weeks of age was only one-tenth that in chickens inoculated at one day of age.

**SUMMARY**

Recent research on the avian leukosis in the areas of electron microscopy, tissue culture and etiologic interrelationships has been reviewed. The specific virus particle of the various leukoses as well as other virus neoplasms are morphologically indistinguishable. It is about 80 μ in diameter, has an electron dense core of 30-40 μ and a distinct limiting membrane which often appears double.

In myeloblastosis, the virus particles arise in viroplasts, also known as "gray bodies"; the latter appear to be derived from mitochondria. Maturation and liberation of the particles are related to the cytoplasmic vesicles and vacuoles. Adenosine triphosphatase activity of the virus is acquired during synthesis in the viroplast and is related to this particular enzymatic activity of the granules of the myelocyte which also are derived from mitochondria. A similar origin of virus particles in erythroblastosis and visceral lymphomatosis has been suggested, but distinct ATPase activity is not involved.

The virus of myeloblastosis has been grown *in vitro* in myeloblasts of normal bone marrow and in the cells of chickens with myeloblastosis. Cultures have been maintained for periods of at least six months and at times have produced 31 particles per cell per hour.

A tissue culture cytopathogenic virus has been obtained from strain RPL12 visceral lymphomatosis and from other sources of this disease. Early results indicated that the virus was identical, or closely related to, the virus causing visceral lymphomatosis. Recent studies indicate that the foregoing should be viewed with question.

In studies on the biological characteristics of various leukosis strains and other sources of lymphomatosis virus, it has been found that all sources of virus which cause visceral lymphomatosis, without exception, also cause other
neoplasms; thus it can be said that “pure” strains of visceral lymphomatosis have as yet not been isolated. Erythroblastosis most commonly occurs as a second neoplasm in chickens inoculated with visceral lymphomatosis material. The proportion of the two leukosis occurring in any group of inoculated chicks is profoundly influenced by the dose of virus. Although there is strong circumstantial evidence that erythroblastosis and visceral lymphomatosis are caused by the same virus and that other neoplasms are caused by different viruses, there are still too many unanswered questions and unexplained experimental results to permit the drawing of any conclusions at the present time.

REFERENCES


RECENT STUDIES OF THE AVIAN LEUKOSIS VIRUSES


A PROGRAM OF IMMUNIZATION AGAINST AVIAN COCCIDIA

AND J. G. KILIAN, D.V.M.

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DEPARTMENT OF VETERINARY MEDICINE

Corvallis, Oregon

Since Johnson (1), and Tyzzer (2), and Tyzzer et al. (3), reported on the immunity produced in chickens following infection with coccidia, and that the immunity was species specific, additional knowledge that would lead to a practical use of this information has been slow in developing. Dickinson et al. (4), reported on a proposed program for immunizing birds against five of the common species of coccidia that infect chickens. Since that report it has been learned, through field trials, that although this program usually gives reasonably good practical results, it fails in enough cases to warrant searching for a more positive means of bringing about immunity to coccidial infection in chickens. Work at the Oregon station has been directed toward this goal and the field results are encouraging.

Field trials have been conducted over a period of more than ten years at the Oregon station. In the early field trials the dosages of coccidia per bird mixed into the feed were approximately those given in the trials reported in 1951 (4), when the coccidia were administered to each bird individually. Clinical "breaks" of varying degrees of intensity were observed in about five percent of the flocks on field trials. It seemed obvious that in such "breaks", for one reason or another, there was not a uniform consumption of the treated feed by the birds. With knowledge of the dosage and time required for the production of immunity of each species as reported by Babcock and Dickinson (5), it was evident that to provide a practical immunity to a large percent of the birds it would require an increased dosage of coccidia to be fed in the feed. The reasoning, that given a small dosage of coccidia the birds would produce many more oocysts which would supply the larger dose for the production of immunity still left too much to chance infection. Even with timing the use of coccidiostatic drug to coincide with the chance heavier infection that might be expected there were "breaks" because on a chance basis the severe infection was delayed and actually struck after the coccidiostat was removed. It has been reasoned, therefore, that one must assure, as near as possible, the exact time of exposure and also assure that the dosage of coccidia that will provide practical immunity will be consumed. To accomplish this, the dosage of coccidia per bird, supplied in the feed, must be such that birds in the brood that will consume only a minimum of treated feed will consume enough coccidia to provide practical immunity. It is recognized that some birds will consume many more coccidia than is needed to produce a suitable immunity. However, since the exact time when the birds have consumed the coccidia is known, it is relatively easy and reliable to provide a suitable coccidiostat to protect the birds against a frank clinical
outbreak of the disease. The hazard in this approach lies in the off-hand chance that a producer may not follow the outlined procedure, and the birds do not receive the coccidiostat at the proper time. Of hundreds of field trials conducted on commercial poultry farms this has never occurred up to the present time.

More than 700,000 chicks have been involved in field trials during the past 10 years. This past year 35 ranches have cooperated in field trials and 97 different flocks of chicks have been raised on these ranches. These trials have involved 154,315 chicks that have been started in 400 different brooders. Mortality and epizoolgy records have been kept on each brood. On all but two ranches there have been no coccidiosis problems. Unexplained clinical "breaks" of *E. acervulina* have occurred simultaneously with severe losses from visceral leukosis. In addition to the coccidiosis the birds had heavy infestations of ascarids and capillaria. With the good success obtained on the rest of the ranches one is prompted to question whether visceral leukosis may have some undisclosed effect upon the coccidial immunity that might otherwise be expected to be present in the birds. Further field studies may indicate whether or not there is a relationship between these serious poultry disease problems.

At present the procedure for immunizing consists of separately preparing cultures of five of the common species of coccidia that infect Oregon chickens. The species used are *Eimeria tenella*, *E. necatrix*, *E. maxima*, *E. acervulina*, and *E. praecox*. The cultures are prepared by separate propagation of each species of coccidia, in susceptible chickens, and then collecting oocyst-laden feces at the height of oocyst production. The oocysts are sporulated by mixing the feces with several times their volume of 2.5 percent potassium dichromate solution. This mixture is stirred and aerated in flasks for three or more days. Then the feces are sieved through a series of sterilized sieves varying from 14 to 250 meshes per square inch. The sporulated oocysts that pass through the sieves are concentrated by allowing them to settle and then pouring off most of the excess liquid.

Estimates of the number of oocysts per ml. in the concentrated cultures are made by making counts of oocysts with a modification of the procedure described by Beach (6). The cultures are also examined for freedom from viable bacteria and fungi. The pure species cultures are then ready for preparing the mixed culture that is made up of the five species that have been individually prepared.

Into bottles containing 200 ml. of one percent potassium dichromate a volume of each culture is added that would provide approximately 2,000,000 sporulated *E. tenella* oocysts, 2,000,000 *E. necatrix*, 1,500,000 *E. maxima*, 20,000,000 *E. acervulina*, 15,000,000 *E. praecox*. This provides a mixed culture preparation for not more than 200 chicks. It is in this manner that the culture is held for ultimate use.

The preferred age for immunizing chicks is from five to 10 days old. The proper dosage (by volume) of mixed culture is mixed with mash at the rate of about one pound of mash for each 100 chicks. The feed mixture is prepared with proper coccidia dosage for each brood of chicks usually within 24 hours from the time it is to be fed to the chicks. All feed should be taken
away from the chicks for two or three hours prior to feeding the coccidia mixed feed. It is important that all of the coccidia mash is eaten and it is also important to provide circumstances that will favor all chicks getting at least one good fill of the coccidia mash. After the coccidia mash has been consumed the chicks are returned to regular starting mash for 24 to 36 hours. Twenty-four to 36 hours after feeding the coccidia mash, the chicks are put on starting mash containing 0.05 percent sulfaquinoxaline (1 lb. per ton) and are fed this coccidiostatic mash continuously for six days. After this period of feeding medicated mash the birds are returned to non-medicated starting mash.

It was self-evident, after several years of field experience, that in order to establish the most satisfactory practical protection against avian coccidiosis certain fundamental factors must be fulfilled.

1. The birds should be in good health and not showing signs of loss of appetite at the time they are fed the coccidia treated mash.
2. The coccidia must be viable and capable of establishing a high degree of infection.
3. The original dosage of each species of coccidia to be given should be sufficient to produce a relatively strong immunity. This dosage will result in clinical signs of coccidiosis if not suppressed by a suitable coccidiostat.
4. The birds to be immunized must have been on a feed containing no coccidiostat prior to the inoculation period.
5. The birds must consume all the coccidia feed and each bird should consume at least one good fill of the coccidia feed. Keeping feed away from the birds for two or three hours usually will make the chicks hungry; however, any feed spilled in the litter must be removed or covered with clean litter.
6. It is essential that there be enough hopper space so that all the chicks can get to the feed.
7. A highly palatable feed should be used for mixing with the coccidia; and a thorough uniform mix of the coccidia must be accomplished.

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1. JOHNSON, W. T.: Immunity or resistance of the chicken to coccidial infection. Oregon Agricultural Experiment Station Bulletin No. 230, 1927.
COCCIDIOSIS IMMUNIZATION AT A POULTRY TESTING PROJECT

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AND S. L. Jamison, D.V.M.‡

Since the development of an official poultry testing project in California in 1939, efforts have been made to establish a uniformity of conditions which would allow an accurate measure of the productive performance of the entries. Methods and schedules of vaccination against Newcastle disease, infectious bronchitis, and fowl pox have been gradually improved and appear to be satisfactory. However, in spite of strict sanitary measures, prophylaxis, and therapy, outbreaks of coccidiosis continued to occur at various stages of the growth and production periods of chickens entered in the random.

### TABLE 1

*Coccidiosis Outbreaks in Recent California Random Sample Laying Tests*

<table>
<thead>
<tr>
<th>Laying Test</th>
<th>Age of Birds When Affected</th>
<th>Type of Coccidiosis</th>
<th>Number of Pens Affected</th>
<th>Mortality†</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th</td>
<td>7-8 months</td>
<td>Intestinal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cecal</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>8th</td>
<td>5 weeks to 1 year</td>
<td>Intestinal</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cecal</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>9th</td>
<td>3-4 months</td>
<td>Intestinal</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cecal</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>10th</td>
<td>6-6½ months</td>
<td>Intestinal</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cecal</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11th</td>
<td></td>
<td>Intestinal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cecal</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Diagnosis of coccidiosis based on examination of dead birds removed from pens.
† Number of dead birds in which coccidiosis was diagnosed.
‡ No evidence of coccidiosis in birds to 9½ months of age.

sample egg laying tests (Table 1). One of the reasons for the lack of control of this disease apparently was the irregularity and varying degrees of exposure to coccidia of birds in the floor pens. It should be kept in mind that the part of the test in which all birds were kept on the floor was comprised of 100 separate pens, each containing 57 birds, at the start. All adjoining pens were separated by solid board partitions, and the entries were divided

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among four separate buildings 75 feet apart. The entry of each breeder was kept in two separate pens, and the two groups were three weeks apart in age. All entries were approximately one day of age when delivered at the project. The irregularity of exposure was further complicated by the presence of at least five immunologically distinct species of coccidia. Under these conditions, a uniform exposure to any one or all of the species of coccidia was not possible. To eliminate this troublesome variable, an immunization program against coccidiosis was believed to be of paramount importance.

Through the cooperation of Dr. E. M. Dickinson of Corvallis, Oregon, a sufficient amount of a suspension of sporulated oocysts containing *Eimeria tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, and *E. praecox* was obtained to vaccinate 5,700 chickens. All of the birds entered in the 11th Random Sample Egg Laying Test received the coccidial suspension at nine days of age. Twenty-four hours later the birds were placed on a 0.05 percent sulfathiazole mash, which was fed for a period of six days in accordance with the recommendations set by Doctor Dickinson (1).

**RESULTS**

No clinical reaction was observed in the chicks following the administration of the coccidia and sulfathiazole treatment.

Although the mortality during the growth period was low, all dead birds were submitted to the State Poultry Pathology Laboratory in Turlock for necropsy and thorough examination. In no instance was coccidiosis diagnosed. A study of the records of previous years indicated that clinical cases of coccidiosis were expected and occurred sporadically or in the form of a mild enzootic during the same period.

On June 24, when the birds from Lot 1 were 112 days old and those of Lot 2 were 91 days old, 50 birds picked at random from 50 pens and representing a cross-section of the entries were brought to the University for challenge against the five species of coccidia. The 50 birds were divided into five groups of 10 birds each and the groups placed in units of an experimental house. Each unit contained five cages, each cage holding two vaccinated birds, one from Lot 1 and one from Lot 2, and a cage with two susceptible control birds obtained from the University normal flock. The latter were eight weeks of age. All of the birds were weighed individually prior to and for the second time on the ninth day following challenge. The feed consumed by the 10 vaccinated birds was determined by weighing the feed which remained in the feedcups and subtracting the amount from the total amount of feed placed in the unit prior to the experiment. The feed consumption of the two control chickens was determined separately.

Each bird of the group was challenged by introducing into the crop a previously determined number of sporulated oocysts which were suspended in one percent sodium dichromate solution. The inocula were obtained from Doctor Dickinson. The challenge dose of oocysts for each species was as follows: *E. tenella*—70,000, *E. necatrix*—43,000, *E. maxima*—150,000, *E.
acervulina—6,000,000, E. praecox—2.4 million. The birds were observed daily for symptoms. At the end of the fourth day the dropping pans were cleaned and the droppings from the fifth through the tenth days were allowed to accumulate to determine the number of oocysts produced by the birds (2). All of the birds were killed and necropsied on the tenth day following challenge.

RESPONSE TO CHALLENGE

E. tenella. As shown in Table 2, the 10 birds showed a considerable degree of immunity as expressed by an increase in body weight and production of a small number of oocysts when compared to the controls. There were no clinical signs nor were there lesions found on necropsy. In only two of the five cages were oocysts found in the droppings.

E. necatrix. The vaccinated group showed an increased body weight over the 10-day period and greater feed consumption than did the controls. No oocysts or signs were observed in the vaccinated group. On necropsy, one of the birds showed a mild congestive enteritis with a few petechiae.

E. maxima. There was a high degree of immunity as demonstrated by the gain in weight of the vaccinated birds, the lack of clinical signs and the absence of coccidial oocysts in the droppings; however, three of the 10 birds showed a slight enteritis or petechiae at necropsy on the tenth day.

E. acervulina. Although the average weight gain was slightly lower than the gains of the other groups of birds, no oocysts or clinical signs were observed in the vaccinated group. An enteritis of a mild nature was found in three of the 10 vaccinated chickens.

E. praecox. This species, which appears to be the least pathogenic of the five types of coccidia, did not produce clinical evidence of an infection, but on necropsy a slight thickening or congestion was observed in seven of the 10 birds. Feed consumption and average weight gain appeared to be within the normal limits.

These results definitely indicate that there was a substantial degree of immunity developed to all five species of coccidia.

The management of the test has reported that this flock was one of the most uniform of any ever raised at the poultry testing project. The mortality of the flock to 18 weeks of age was 0.8 percent, and the highest mortality of any single entry was only 4.3 percent. The general appearance of the pullets was excellent. At 23 weeks of age, Lot 1 birds were rapidly coming into production, and nine of the 50 entries had reached 50 percent production for at least one day. A continued careful examination of birds dying from all causes has not revealed a clinical case of coccidiosis to date. The birds are 38 and 41 weeks of age at the time of this report.

It is anticipated that successful immunization against coccidiosis will help make possible a better evaluation of productivity of entries in the random sample laying tests.
### TABLE 2
**Results of Challenge of Birds Approximately Three Months Following Immunization With Five Species of Coccidia**

<table>
<thead>
<tr>
<th>Challenge Species</th>
<th>No. Oocysts per Dose</th>
<th>No. Birds</th>
<th>Feed Consumed per Bird (lbs.)</th>
<th>Av. Wt. Gain or Loss (lbs.)</th>
<th>Oocysts per gm. Droppings Collected 5th thru 10th day</th>
<th>Signs</th>
<th>Necropsy (10th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. tenella</td>
<td>70,000</td>
<td>10*</td>
<td>1.29</td>
<td>+0.17</td>
<td>8,564$</td>
<td>None</td>
<td>NVL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2†</td>
<td>1.51</td>
<td>-0.19</td>
<td>1,430,000</td>
<td>Both slightly depressed 6th day. Tr. blood droppings 6th day. Emmaciated, NVL in one bird. NVL in second.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susc. Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. necatrix</td>
<td>43,000</td>
<td>10*</td>
<td>1.37</td>
<td>+0.15</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2†</td>
<td>1.19</td>
<td>+0.04</td>
<td>536,000</td>
<td>Both slightly depressed 6th day. Mkd. thickening intestine in one bird. Catarhal and hemorrhagic enteritis in 2nd. Cecal contents blood tinged in both.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susc. Controls</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>E. maxima</td>
<td>150,000</td>
<td>10*</td>
<td>1.21</td>
<td>+0.18</td>
<td>0</td>
<td>None</td>
<td>Slight enteritis or petechiae 3/10.</td>
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<td></td>
<td></td>
<td>Vaccinated</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2†</td>
<td>1.37</td>
<td>-0.11</td>
<td>103,189</td>
<td>Both slightly depressed on 6th day. Mucus and blood in droppings. Mod. number of petechiae and sl. ballooning of intestine in one bird. Slight hem. enteritis in 2nd.</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>Susc. Controls</td>
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</tr>
<tr>
<td>E. acervulina</td>
<td>6,000,000</td>
<td>10*</td>
<td>1.25</td>
<td>+0.08</td>
<td>0</td>
<td>None</td>
<td>Trace to mod. enteritis 3/10.</td>
</tr>
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<td></td>
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<td>Vaccinated</td>
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<tr>
<td></td>
<td></td>
<td>2†</td>
<td>1.38</td>
<td>-0.07</td>
<td>762,010</td>
<td>Both slightly depressed 6th day. Mild catarhal enteritis in one bird. Mod. catarhal enteritis with petechiae upper third in 2nd.</td>
<td></td>
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<tr>
<td></td>
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<td>Susc. Controls</td>
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</tr>
<tr>
<td>E. praecox</td>
<td>2,400,000</td>
<td>10*</td>
<td>1.39</td>
<td>+0.21</td>
<td>779$</td>
<td>None</td>
<td>Mod. thickening of intestine in one bird. Tr. thickening of intestine in 2nd.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaccinated</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2†</td>
<td>1.58</td>
<td>+0.18</td>
<td>619,264</td>
<td>None</td>
<td></td>
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<tr>
<td></td>
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<td>Susc. Controls</td>
<td></td>
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* Group composed of equal number of birds 13 and 16 weeks of age. One bird of each age placed in each of five cages.
† Controls, 8 weeks of age.
‡ Droppings in 3 cages contained no oocysts.
§ Droppings in 2 cages contained no oocysts.
mkd. = marked; mod. = moderate; NVL = no visible lesions; sl. = slight; tr. = trace.
ACKNOWLEDGMENT

The authors wish to express their appreciation to the members of the Poultry Improvement Commission of the State of California, and particularly, Mr. E. A. Johnson, Superintendent, Mr. Howard Lusk and Ralph Len for their assistance and cooperation which made this study possible.

REFERENCES


REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

R. A. BANKOWSKI, Chairman, Davis, California; A. CHRISTIE, Kingston, New Hampshire; E. MILTON DICKINSON, Corvallis, Oregon; R. F. GENTRY, University Park, Pennsylvania; HARRY E. GOLDSTEIN, Columbus, Ohio; N. O. OLSON, Morgantown, West Virginia; R. E. OMOHUNDRO, Washington, D. C.; B. S. POMEROY, St. Paul, Minnesota; CHARLES S. ROBERTS, Auburn, Alabama; ROBERT H. SINGER, Frankfort, Kentucky; WILLIAM L. SIPPEL, Kissimmee, Florida; W. R. TEETER, Dover, Delaware; J. FRANKLIN WITTER, Orono, Maine.

Although the volume and intensity of research on problems affecting poultry has markedly increased during the past decade, the problems and economic losses to the industry have kept pace accordingly. This was emphasized by the revelation of the serious losses suffered by the industry through condemnations upon the mandatory federal poultry meat inspection, which was initiated and put into effect on January 1, 1959. A high percentage of the losses was due to infections of the respiratory tract. Although recommendations to increase research activities for prevention of high condemnations were made, causes for a number of diseases resulting in poor carcasses are well established. A concentration on an educational program and dissemination of information should be made which would considerably improve the situation and insure sound, wholesome, and unadulterated products for interstate shipment.

The Committee wishes to reiterate its statement of last year's report regarding multiple infection of the respiratory tract in relation to the overburdened vaccination schedule with the use of virulent infectious agents in the present-day vaccine. The Committee also wishes to emphasize that viruses may undergo profound alterations in their antigenic constitution, which has been exemplified in infectious bronchitis and Newcastle disease. Because this possibility is a real challenge to the industry and to manufacturers and investigators of immunizing agents, concentration of efforts on the improvement of the immunizing agents and initiation of eradication programs should be stressed.

RESPIRATORY DISEASES

A number of outbreaks of Newcastle disease have been reported from the Southeastern Coast of the United States. There is no single explanation for the cause of these breaks in the disease; however, the following may be contributing factors: (1) the lack of vaccination against the disease, (2) inadequacy of some strains of ND virus presently used as live virus vaccines to immunize against some field strains, (3) failure to vaccinate according to the recommended procedure or schedule. These outbreaks were also
accompanies the provocative or triggering of respiratory diseases concurrent with ND in many areas. Investigations also reveal the continuation of the substitution of immunization procedures for proper husbandry and management practices.

Newcastle Disease. "Breaks" in poultry flocks vaccinated with commercial Newcastle disease (ND) vaccines probably have no single cause. An appreciable number of flock owners do not always follow directions or recommendations for administering a given product for maximum protection, LaRose and Van Roekel (1) concluded from a study of a number of field flocks vaccinated for ND and infectious bronchitis (IB). Another factor may be found in a study by Hanson and Alberts (2), who showed that IB vaccine given simultaneously with ND vaccine interferes with the response of the latter. Interference between the two agents was operative for 16 to 21 days. The reaction appeared to be nonspecific and is not explained by the mechanism of live viral interference. A significant role in vaccination failures in poultry may also be played by viral sensitization and true interference between viruses, as demonstrated in previous years and with other agents.

Still another factor that may interfere with a desired response to vaccination is indicated in the work of Cole and Hutt (3). Genetic strains of chickens differed in their reaction to an ND wing web vaccination: one strain showed a significantly higher (five percent against 0.7 percent) mortality in the immediate post-vaccination period and a higher subsequent mortality (seven percent against 1.5 percent) from diseases of the respiratory system. The authors also demonstrated an increase in frequency of ovulation in nonlayers in the more susceptible strain of birds.

A formidable factor is the condition of the vaccine (4). Hejl and Faber (5) reported on an improved membrane filtration method of detecting contaminants in antibiotic-treated live virus vaccines. A study of 53 commercially available ND vaccines proved that 35 were contaminated, of which one-third could not be detected by conventional procedures. A number of products were grossly contaminated and considered unsuitable for marketing. The authors recommended that vaccines should be prepared more carefully and tested for contaminants before the addition of antibiotics.

Legenhausen and Sinkiewicz (6) evaluated two killed Newcastle disease vaccines prepared from the GB (Texas) strain of NDV. A single dose of 1.0 ml of a commercial-oil-emulsified ultraviolet irradiated or a crystal-violet ethylene-glycol inactivated vaccine given to passively immune chicks at ten days of age produced no effective immunity to the disease upon challenge at six weeks of age. Birds that received two doses of either vaccine at the rate of 0.5 ml at ten days of age and 1.0 ml 32 days later exhibited resistance to intramuscular challenge throughout a 13-month observation period. The investigation expressed the duration of immunity following vaccination with the killed product in terms of serology and intramuscular challenge, without reference to protection against a drop in egg production. Small doses (0.25 and 0.5 ml) of crystal-violet-inactivated vaccine at short intervals failed to
TRANSMISSIBLE DISEASES OF POULTRY

offer any advantage over a single injection of the same quantity (7). Doses of 1.0 to 2.0 ml significantly increased resistance to challenge, particularly as the intervals between injections were increased to one, two, three, and four weeks. The greater immunity following booster injections with the higher dosages could be demonstrated serologically. In these experiments, the duration of protection from the varied dose schedule was not measured. Gill et al. (8) investigated the comparative value of three adjuvants—(1) crystal-violet ethylene-glycol, (2) aluminum hydroxide jell, and (3) phosphorolated hesperidan—on the antigenicity of an inactivated vaccine. All three adjuvants were similar in maintaining immunogenicity of virus given at 14 days and repeated at 12 weeks of age. Challenge with the homologous and heterologous strains of NDV showed a high degree of resistance to signs and death until the birds were 32 weeks old.

In contrast to studies conducted with inactivated vaccines to produce a substantial and durable immunity in chickens, Bankowski et al. (9) continued to study the immunogenicity of an avirulent living-tissue culture-propagated NDV vaccine (TCND). In a comparative study neither TCND nor the inactivated vaccine was shown to spread from vaccinated to susceptible pen contact controls during a 54 week period. Both vaccines offered protection against signs of disease and death following challenge, but the TCND protected layers against a drop in egg production whereas the inactivated vaccine did not. The experiments also indicated that there was little or no correlation between detectable serum antibodies and protection offered to the respiratory epithelium and reproductive systems following challenge. The most sensitive gauge of degree and duration of immunity to ND was found to be the protection afforded to the reproductive tract. Other evidence indicated that the respiratory and reproductive tracts may react independently to a challenge dose of virulent virus. A further report (10) demonstrated that TCND given at five and 14 weeks of age protected chickens, following challenge intramuscularly or by contact with experimentally infected birds, against a drop in egg production when the vaccinated chickens reached 101 weeks of age. The TCND virus was readily propagated in pig kidney cells. Such cultures produce titers of $10^7$ and $10^8$ per ml and are presently used for the production of vaccine for immunizing chickens for field trials.

Basic research with ND virus is continuing. Zuschek et al. (11) demonstrated that three strains of ND virus (B1, GB Texas, and Roakin) were propagated at temperatures between 33°C and 45°C in chorio-allantoic membrane (CAM) suspended in Tryode's solution; all three strains grew best at 42°C. In another report, the same authors (12) studied the influence of several compounds on the growth of NDV in vitro. From a limited number of trials Durand and Eisenstark (13) suggested that NDV could be titrated in tissue culture by detecting color changes resulting from differences in the metabolism of infected trypsinized chicken embryo cells.

In contrast to previous observations, Dardiri et al. (14) were unable to demonstrate that the aqueous humor of chickens was a reservoir for Newcastle disease virus. These authors readily isolated the virus from the aqueous humor of naturally infected chickens showing respiratory signs, but not from
birds showing nervous symptoms or from birds with a high degree of immunity.

*Laryngotracheitis* (LT). Since changes or variations can be expected in any biological process, variations in the behavior of etiological agents should be expected in the ever-changing virus and poultry populations. Cover and Benton (15) studied an isolate (labeled 146) obtained from a diseased flock showing respiratory distress and lesions unlike those of chronic respiratory disease (CRD). The agent produced typical intranuclear inclusions in infected tracheal epithelial cells, and there was no difference in the antigenicity of the isolate from several LT virus strains tested. Minor differences were noted in clinical response of the host to the agent. Intranasal administration of the agent resulted in no signs of respiratory distress other than a transient respiratory reaction during the sixth to the eighth days. Upon challenge four weeks later the immune response was equal to that in birds vaccinated with a commercial product by the conventional method. Infection of birds by atomization of the 146 virus resulted in severe adverse reaction, characterized by an acute respiratory distress and a mortality. The reaction was influenced by the virus concentration in the inoculum and, to some extent, with the age of the birds exposed (16).

Armstrong (17) reported that scrapings of the tracheae of suspected cases of laryngotracheitis, when stained and examined microscopically for inclusion bodies, proved to offer a rapid and consistently reliable procedure for diagnosis of LT. The test was particularly effective for detecting early clinical stages of the disease and identifying atypical forms of the infection. Watrach et al. (18) studied the structure of LT virus and the cellular changes that occurred in infected CAM. The virus inclusions varied considerably in size, from one to 20 μm. Electron microscope studies revealed that the virus particles were seen most commonly in the cytoplasm and rarely in the nucleus. The virus particle consisted of a dense core, a mid-zone of lesser density, and an outer capsule. It was apparent that the virus is more complex than previously believed, which may account for the diversity of previous reports on its average size.

**Infectious Bronchitis.** Propagation and demonstration of the cytopathogenic effect (CPE) of the Beaudette strain of infectious bronchitis virus in tissue culture added another tool for study of this disease agent (19). The CPE was arrested by specific antisera but not by normal chicken serum. The latter observation may result in a more rapid, simpler, and less costly neutralization test than the present chicken embryo technique. It was interesting to note, however, that the tissue-culture-propagated virus was neutralized by both the Massachusetts and Connecticut type antisera in the tissue culture system, though only the Massachusetts type antiserum neutralized the homologous virus in the chick embryo system.

Another lead toward a simpler and more rapid method of identifying IB virus was demonstrated by Corbo and Cunningham (20). The authors showed that IB-infected aaf treated with trypsin caused an agglutination of chicken red blood cells. Apparently, the enzyme-modified virus provided
a product that was adsorbed to the cell surface, causing hemagglutination. Though this could not be repeated with all strains of IB, hemagglutination with the adaptable strains was found to vary directly with virus concentration. Raggi and Raymond (21) were unable to differentiate among six strains of commercial IB virus by studying the death pattern in chick embryos following a single or a number of serial passages through embryonating eggs. The Massachusetts strain of IB was found to be stable in triple-distilled water buffered to pH 6.39, 7.19, or 7.85 and held at 25°C for at least 24 hours (22).

Chronic Respiratory Disease and Infectious Sinusitis. In an attempt to classify the status of CRD, Chu (23) has differentiated between the specific chronic respiratory disease of chickens and chronic secondary complication following one of the virus infections. The agent for specific uncomplicated CRD is the Mycoplasma gallinarium (MP) organism usually designated as PPLO. Isolation of this organism is still employed as a diagnostic aide, but is not completely reliable since the media generally used are apparently not satisfactory for the isolation of pathogenic strains from all cases (24). Workers in India have reported the isolation of MP from 114 out of 114 birds showing clinical evidence of CRD (25). The failure to identify these as pathogenic or non-pathogenic strains does not give a true evaluation of the type of infection present. Many of the strains are probably of the non-pathogenic type and some possibly should be identified as bacterial L-forms (26).

The type of lesions produced in turkeys by the pathogenic strains of MP is well known. A keratoconjunctivitis has been reported as possibly associated with MP infection (27). However, only one turkey was infected; the type of MP organism involved was not indicated, and reproduction of the condition was not possible.

Extensive egg transmission studies in chickens were carried out by Fabricant et al. (28). The recovery of MP from the eggs of naturally and artificially infected hens was of very low incidence. No isolations were obtained from non-fertile eggs. The number of isolations from fertile eggs varied greatly with the effect on embryonic viability being much more severe in birds in full production. The reisolation of MP from artificially infected eggs is significantly less in the groups that have been dipped in erythromycin solution (29). The egg transmission of MP in turkeys, however, is more easily measured. High percentage of poults from infected supply flocks show sinusitis outbreaks, while supply flocks having no clinical evidence or history of infectious sinusitis usually remain free of the disease (30). The artificial infection of non-infected breeding flocks with MP was not effective in producing consistent transmission of the disease (31). In turkey breeder females in which infection was established, typical clinical symptoms were evident. Egg production and hatchability were drastically reduced and the resulting infected progeny developed persistent clinical infectious sinusitis (32). Three of five turkey strains and two of seven chicken strains of MP caused grossly visible sinusitis in turkeys (33). The other strains had either
lost their pathogenicity during serial passage in carbohydrate media or were originally of the non pathogenic type. These results helped to establish the relationship of turkey sinusitis and CRD in chickens. When serial exudate and broth cultures were inoculated intracheally into regular or germ free turkeys, an aesarosacitis was produced (34). Filtrates of these inoculums failed to produce lesions.

The role of pathogenic MP in CRD of chickens is still not definitely established, but there is little doubt of their association with infectious sinusitis of turkeys. In one instance, an outbreak of sinusitis in turkeys was transmitted to a flock of pheasants, producing a marked thickening of the eyelid, swollen infraorbital sinuses, and a mortality of about 30 percent (35). Isolations of MP were made which were used to reproduce the lesions and symptoms in pheasants and turkeys similar to the natural outbreak.

Prevention of the CRD complex by testing for the presence of MP and the examination of pipped embryos was reported (39). The complexity of this disease was emphasized. MP were considered to produce a mild infection which is not of economic importance. Subsequent exposure to respiratory virus reduces the host resistance which is followed by the invasion of secondary bacteria. The condition with severe lesions and mortality then becomes of economic importance.

The detection of MP by serological testing has been used with CRD outbreaks (36, 37) and with infectious sinusitis (38). Most of these involved the use of the serum plate test which was beneficial in the detection and evaluation of flock infections but was not dependable for individual bird diagnosis.

Attempts to control MP infection and CRD included the spraying of chicks at one day and 3½ to four weeks of age with dihydrostreptomycinpropyleneglycol (39). Such treatment reduced mortality only slightly but showed a significant improvement in weight gain. In Vitro studies using streptomycin, furazolidone, chloromycetin, and tetracycline showed they were all capable of suppressing the growth of MP (40). However, streptomycin and furazolidone exhibited this effect only after an initial lag phase.

Treatment of infectious sinusitis has been successful by the use of erythromycin (41, 42). The only effective route of administration was directly into the swollen sinus after a portion of the exudate had been removed. Implantation or feeding of the antibiotic was not effective.

Infectious Coryza. Diagnoses of infectious coryza have decreased sharply during recent years. The disease is being reported primarily on the west coast, where in the pure form, it is a minor problem. It is more frequently reported in combination with other infections of the respiratory tract. A method for presumptive diagnosis consists of making a smear of the sinus exudate and, after staining, is examined for the characteristic slender filaments that are often beaded or granular. The diagnosis can be confirmed by inoculation of the exudates into the sinuses through the palatine cleft of susceptible birds. A mucopurulent exudate with or without a facial edema
TRANSMISSIBLE DISEASES OF POULTRY

develops in 24 to 72 hours in which myriads of organisms can be demonstrated.

It is felt that Hemophilus gallinarum infection has reached a low ebb due
to the inclusion of antibiotics in poultry feeds and due to the fact that many
times the infection is masked by a primary virus infection.

A search of the available current literature has failed to reveal any papers
on this subject during the past year.

INFECTIOUS SYNOVITIS

To test immunity to infectious synovitis (IS) 178 survivors following
inoculation with the Bridges strain of IS were challenged. Only 23 birds
survived. If immunity was obtained, it was slight and only after severe
infection. No immunity was demonstrated by the use of formalized vaccines.

Serum neutralization and plate agglutination tests failed to demonstrate any
antibodies (43). Leece (44) reported the isolation of Mycoplasma (MP)
from three strains of the IS agent. Swine serum, 10 percent CO₂ and a
staphlococcus streak was required for growth on agar plates.

The IS agent was present in the blood eight hours after IV inoculation
and 48 hours after inoculation. The agent persisted for 15 days but
not for 52 days. The agent was not demonstrated from internal organs of
birds which had received the agent 100 or more days previously (45).

Pheasants and geese, but not ducks, were susceptible to IS. The throm-
bocyte, heterophil, monocyte and total leukocyte counts and sedimentation
rates of infected birds were greater than in control birds. The hematocrit,
hemoglobin content, erythrocyte and lymphocyte counts were less. The signs,
lesions and histopathology of IS were described (46).

A difference in susceptibility to antibiotics of strains of the IS agent was
shown. Some strains of the agent were susceptible to chlortetracycline (CTC)
(50 gm/ton of feed) whereas some were not affected until the CTC dosage
was increased to 200 gm/ton. In all trials the order of efficacy of drugs as
therapeutic agents was CTC, oxytetracycline (OTC) and furazolidone (nf-
180). No differences in efficacy of the antibiotics was shown as the size of
the inoculum to produce the disease was increased (47). However, other
workers have shown that the virulence or size of the inoculum or both affected
the efficacy of antibiotics as a control measure of IS (48, 49).

Infectious synovitis has been used by several workers to evaluate the
biological activity of the tetracycline antibiotics potentiated by low calcium
diets and terephthalic acid or both (48, 50, 51). The antibiotics
were potentiated two to four times by terephthalic acid (TPA) and
approximately two times by a low calcium ration (0.47 to 6 percent). Even
on a low calcium ration TPA potentiated CTC and OTC (50, 51, 52). The
magnesium level in the ration also influenced efficacy of CTC (52).

INFECTIOUS HEPATITIS

A viral agent less than 300 millimicrons in size was isolated from a highly
fatal disease (Hepatitis) of poults occurring during the first two weeks of
life (53, 54). The lesions were characterized as a focal degenerative hepatitis hepatic congestion or hemorrhage. Degenerative changes were found in the pancreas. Chicks were refractory to the agent. The disease was found in Canada and the United States.

Vibronic avian infectious hepatitis continues to be a problem. The diagnostic laboratories in some states reported vibronic hepatitis in two to six percent of their consignments from one state (55). The diagnosis of vibronic hepatitis is based on the isolation of the causative agent in five to seven day old embryonating chicken eggs and subsequent identification of the agent by its cultural and morphological characteristics (56). Chlortetracycline, oxytetracycline and furazolidone at 200 to 400 grams per ton of feed significantly suppressed development of the disease process (56).

**ERYSIPelas**

_Erysipelothrix rhusiopathiae_ infection in turkeys appears to be endemic in some of the heavy turkey producing areas of the United States. The disease has yielded to a program of inoculation with an _E. rhusiopathiae_ bacterin in areas where it commonly has reached epizootic proportions during the fall and winter months. The common practice is to inoculate subcutaneously in the neck region when the young birds are 12 to 14 weeks old. This protects most birds up to slaughter age of five to six months. If breeders are to be selected from such flocks, they should be given a second inoculation since the immunity from the first injection appears to deteriorate after 60 to 90 days.

It is important that diagnosticians be alerted to the increased number of reports of _E. rhusiopathiae_ infection occurring in chickens (57).

**AVIAN LEUKOSIS COMPLEX**

The majority of published reports on the avian leukosis complex that have appeared in the literature since the last report of this Committee have dealt with cultivation and characteristics of the virus.

Cultivation of the virus of avian lymphomatosis in chick embryo liver cells was reported by Sharpless _et al._ (58). The agent induced in 12-day-old chick embryos a disease histologically resembling lymphomatosis. In chicks it induced lymphomatosis in 20 to 50 percent of the birds in nine months, and an agent was recovered that was similar to the original agent. Fontes _et al._ (59) reported serial passage of an agent in embryo liver tissue from RPL 12 infected chickens. The virus produced a marked cytopathological effect. Neutralization tests with antiserum against RPL 12 indicates a relationship of tissue culture with RPL; however, positive identity of the cytopathogenic agent with the virus causing visceral lymphomatosis was not established. Davis _et al._ (60) also propagated the virus of avian visceral lymphomatosis in cultures of normal chicken embryo spleens for ten three-day serial passages. _In vitro_ cultures of neoplastic tissue from chickens affected with avian visceral lymphomatosis cultures showed marked proliferation and migration of lymphoblasts. Supernatant fluids from cultures of the
neoplastic chicken tissues and Silas 03 filtrates of the fluids produced typical avian visceral lymphomatosis in a high percentage of inoculated birds; however, chickens which were inoculated with tissue culture material containing avian visceral lymphomatosis virus did not show evidence of neoplastic disease. At 38 days they were resistant to challenge with highly virulent tumor material.

Avian osteopetrosis was induced in normal birds by Homes (61) following intraperitoneal injection of day-old chicks with whole blood, bone marrow or unfiltered plasma from birds with the active disease. The author stated that these observations indicate that a transmissible agent is concerned in the etiology of osteopetrosis in the fowl and suggest that in field cases the egg may be one mode of transmission.

Avian lymphomatosis virus propagated in chick embryo liver tissue cultures was studied by electron microscopy (62). Identification of the infectious agent was made by two methods and the size of the infectious particles was found to be about 90 millimicrons and appeared to be spherical. The number of particles per tissue culture infectious dose was about five for one of the two virus strains and 35 for the other. Bernhard et al. (63) examined ultrathin sections of pellets formed by ultracentrifugation of plasmas from birds with myeloblastosis and erythroblastosis using the electron microscope. Structures were observed in material from both diseases that were similar in appearance and represented by images of round or oval shape consisting of a dense core of about 48 millimicrons diameter. The total particle diameter was reported to be about 80 millimicrons.

Degeneration of the lens with typical cataracts was reported as occurring in chickens with lymphomatosis (64). The lesions in the lens were described.

ORNITHOSIS

Only one outbreak of ornithosis in turkeys was reported during the past year. The disease occurred in a flock of 600 breeders in which symptoms of a drop in egg production and a 10 percent mortality occurred (Minnesota). The flock was placed on chlortetracycline for three weeks and slaughtered under close veterinary supervision three months following the diagnosis.

A number of reports relative to basic understanding of the disease in turkeys have appeared in the literature. Page (65) traced the path of the disease agent in turkeys following exposure by the air-borne and oral routes. The highest concentration of the agent was found in the lungs, air sacs, and pericardium. The tissues of recovered birds apparently were sterilized over a period of two months. However, such birds were capable of excreting the agent into the environment, thus constituting a source of infection. Moore et al. (66), experimentally infected herons and egrets and found them to be relatively resistant to the highly virulent JO strain of ornithosis. Although two of the 16 birds in the experiment died, those remaining were apparently normal, developed serologic ICF titers, and intermittently shed the agent for at least 67 days. A further report on the pathogenicity of six strains of ornithosis and psittacosis virus in mice suggested the use of this animal for
determining the pathogenicity index of some agents (62). A study of the stability of an ornithosis agent in a commercially processed turkey indicated that the agent survived in the carcass for at least 372 days of storage at $-20^\circ \text{C}$ or below. It was also shown that normal roasting temperatures and times recommended in cook books were sufficient to destroy the agent. The agent, however, is not unduly resistant since a 20 percent mammalian tissue suspension heated to $56^\circ \text{C}$ destroyed the agent in less than five minutes; to $37^\circ \text{C}$, in less than 48 hours; to $20^\circ \text{C}$, in less than 12 days (68).

Detailed studies have been reported on three outbreaks which occurred in California in 1954 through 1958 (69, 70). In view of the differences shown by agents of low virulence to laboratory animals, a study of the reaction of mice and susceptibility of agents of low virulence to antibiotics was reported (71, 72).

Donaldson et al. (73), demonstrated that a diagnosis of ornithosis can be made rapidly by culturing tracheal and cloacal specimens in yolk sac explants and observing the explants with the aid of fluorescent antibody. A comparative study of the tissue culture and immunocytochemical findings with indirect complement fixation titers indicated that the former may be a more reliable means of diagnosis. Furthermore, the technique is sufficiently simple and safe and can be conducted by inexperienced personnel. The histopathologic changes have been studied in a group of over 500 turkeys infected with ornithosis (74).

**SALMONELLOSIS**

**Pullorum Disease**

The voluntary programs of the National Poultry and Turkey Improvement Plans continue to show progress in the reduction of the incidence of Pullorum disease (75, 76). More than 38 million birds, 95.6 percent of the total, were in flocks qualified as United States Pullorum-Typhoid Clean. The number of participating hatcheries as well as flocks has declined over the previous year, but the average flock size and total number of birds in participating flocks have increased as well as hatching egg capacity of participating hatcheries. 63.8 percent of the hatcheries and 75.8 percent of the hatching egg capacity in the country are under the NPIP.

The same trend that has occurred under the NPIP has happened under the turkey plan. The flocks are increasing in size and becoming fewer, but the capacity of participating hatcheries has increased. For the first time all turkey flocks under NTIP qualified for the United States Typhoid Clean classification.

The results of the program are indicated in Table 1.

The development of an inter-state regulation by the Animal Disease Eradication Division, United States Department of Agriculture, is under consideration and will be considered by the industry.

Because pullorum disease has been reduced to a low incidence, less emphasis has been placed on hatchery sanitation programs. Wright et al. (77) have reported on their work in Canada to develop a method for determining
the microbial population of incubators and thus improve the sanitation program in the hatchery.

There is interest in the development of a model program for the eradication of pullorum disease and fowl typhoid on an area basis. The North Central Poultry Disease Conference at its 1959 meeting at Ames, Iowa, in June encouraged the development of such a model program.

The percentages of chickens and turkeys tested by the various methods, 1950 and 1958, indicate that 31.28 percent of the chickens were tested by the tube method as compared to 68.28 percent by the whole blood method in 1958 as compared to 27.58 percent and 70.02 percent respectively in 1950. The serum plate test was used on the remainder of the birds (78).

**PARATYPHOID**

During 1959 at least two states (Minnesota and Iowa) have developed a *S. typhi-murium* control program that offers official recognition to the flock and hatchery operating under the voluntary program.

Huey and Edwards (79) reported on the study of strains of *S. typhi-murium* isolated from man and animals before 1948 and an equal number of strains isolated after 1956 as to their resistance to antibiotics. Significant results were obtained only in the case of the tetracyclines and recently isolated strains.

Recent reports (80, 81, 82, 83) have re-emphasized the importance of Salmonella contaminated feedstuffs in the dissemination of Salmonella serotypes to livestock and poultry. An English report (80) involved the bacteriological examination of 1,262 samples of animal feeding stuffs and organic fertilizers with the isolation of 88 Salmonella serotypes. Boyer *et al.* (81) isolated nine serotypes from turkey feed and meat scraps. Watkins *et al.* (82) examined 200 samples of animal by-products and found 18.5 percent contaminated with Salmonellas. Twenty-eight serotypes were isolated. Recontamination of the cooked product in the processing plant appeared to be the source of the infection.

Grady and Pomeroy (83) in 1959 examined 504 samples of animal and poultry by-products submitted by quality control laboratories of feed companies and found 23.2 percent of the samples contaminated with Salmonellas. Thirty-seven serotypes have been identified.

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**TABLE 1**

*Chickens and Turkeys, Officially Tested for Pullorum Disease, Number and Percent of Reactors, 1956-1959*

<table>
<thead>
<tr>
<th>Year</th>
<th>Chickens Tested</th>
<th>Reactors</th>
<th>Turkeys Tested</th>
<th>Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Reactors</td>
<td>Number</td>
<td>Reactors</td>
</tr>
<tr>
<td>1956</td>
<td>36,112,781</td>
<td>25,529</td>
<td>3,310,470</td>
<td>1,014</td>
</tr>
<tr>
<td>1957</td>
<td>40,614,440</td>
<td>18,369</td>
<td>3,828,755</td>
<td>2,170</td>
</tr>
<tr>
<td>1958</td>
<td>36,756,760</td>
<td>11,423</td>
<td>3,509,580</td>
<td>640</td>
</tr>
<tr>
<td>1959</td>
<td>40,716,266</td>
<td>9,920</td>
<td>3,940,033</td>
<td>437</td>
</tr>
</tbody>
</table>

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0.07
0.045
0.03
0.024
0.03
0.06
0.018
0.01

The percentages of chickens and turkeys tested by the various methods, 1950 and 1958, indicate that 31.28 percent of the chickens were tested by the tube method as compared to 68.28 percent by the whole blood method in 1958 as compared to 27.58 percent and 70.02 percent respectively in 1950. The serum plate test was used on the remainder of the birds (78).
REPORT OF COMMITTEE

AVIAN ENCEPHALOMYELITIS

Reports from 20 diagnostic laboratories, mostly along the Atlantic seaboard but extending through the West to California and representing 16 states, indicate that 16 laboratories believe the importance of AE is remaining about the same, two feel it is diminishing and two increasing. Six reported the use of AE vaccine to a limited extent on a trial level. No other controls of any consequence are in use. Research is increasing with seven laboratories reporting some investigations in progress. Some of these use field trials with the vaccine. Michigan and Ohio are conducting extensive field trials. Within another year the results should enable a more precise evaluation of the vaccination of replacement stock as a means of preventing outbreaks in brooder chicks.

COCCIDIOSIS

It is well understood that poultry raising methods evolved during the past 50 years have served to promote the development of avian coccidia. The continued concentration of more and more birds on litter in smaller and smaller space has provided conditions for a rapid cycle of coccidia development. It has shown that by restricting the dosage of coccidia ingested one can prevent clinical signs of coccidiosis from developing. This was first accomplished by various management practices. Since Herrick and Holmes (84) demonstrated the coccidiostatic effect of sulphur, there has been a succession of drugs discovered that may be considered effective coccidiostats when properly used. McLoughlin and Chester (85) have shown there may be some slight relative differences between some of these agents but, from a practical sense when properly used, most coccidiostats may be expected to be helpful in reducing the incidence of coccidiosis. In spite of the many good coccidiostats available, coccidiosis is still one of the major causes for loss to the poultry industry.

PARASITIC DISEASE

It was reported by Shumard et al. (90) that hygromycin B given in the feed at the rates of eight and 10 Million Units per ton very effectively controlled Ascaridia spp. and Heterakis gallinae when fed for a four weeks period. The effective prevention of infection by the two nematodes by the use of hygromycin was also demonstrated.

Dixon et al. (86) compared the effectiveness of feeding hygromycin B, hygromycin B and phenothiazine NF in combination, and phenothiazine NF alone in the control of Ascaridia galli and Heterakis gallinae in broilers. They found hygromycin alone or in combination with phenothiazine to be most effective. The value of the combination as an anthelmintic is that along with the reduction of the parasite burden, there is also a reduction in viability of the parasite ova thereby reducing the contamination of the environment.

Reid (88) studied the effects of temperature on the time required for eggs of Ascaridia galli to develop from the one celled stage to an infective larva.
He found that the minimum time required was five days at 34°C. He noted that litter temperatures under broiler house conditions may often be as high as 37°C due to habitual hovering of birds in certain areas.

An inter-relationship between Hypovitaminosis A and Ascaridia galli infestation in poultry was demonstrated by Pande et al. (87). The condition of Hypovitaminosis A favored infestation with Ascaridia galli while the latter, in turn, by damaging the intestinal mucosa predisposed the birds to a state of Vitamin A deficiency.

Reid (89) summarized in table form and with photomicrographs the principal morphological characteristics of the eight common species of chicken tapeworms as an aid in their differentiation. The knowledge gained by specific identification of poultry tapeworms enables control measures to be directed against specific intermediate hosts.

**MISCELLANEOUS**

Of considerable concern to the poultry industry has been the widespread occurrence of fowl cholera. A fowl cholera vaccine was developed by the Animal Disease and Parasite Research Division of the United States Department of Agriculture and is produced commercially. Optimum protection has not always been obtained since this product cannot be used after the disease has appeared, or when given to a flock that is not in good condition at time of vaccination. Experimentally the vaccine was proven to be of great value up to nine months following vaccination.

Several outbreaks of spirochetosis were diagnosed in flocks in Arizona in the spring and early summer of this year.

In conjunction with the 1958 AVMA convention the American Association of Avian Pathologists met to ratify the constitution, and arrangements were made for incorporation of the AAAP in the State of Delaware. With nearly 100 members, and arrangements made to assume the responsibility for publishing the journal "Avian Diseases," the organization looks forward to an increasingly active future under the leadership of the officers assisted by a board of four directors. Special committees will undertake studies and specific activity, such as a survey of the adequacy of teaching veterinary students in the field of poultry, cooperation with other organizations to improve mortality and morbidity statistics, a study of the feasibility of pullorum disease eradication, improving liaison with organizations allied in interest to the AAAP and associated with poultry production.

The manual, "Methods for the Examination of Poultry Biologics," has been published by the National Academy of Sciences—National Research Council. It is a report of the Poultry Disease Subcommittee of the National Research Council's Committee on Animal Health in cooperation with the regional technical committees on respiratory diseases of poultry of the agricultural experiment stations. It is available from the Office of Publications, National Academy of Sciences—National Research Council, 2101 Constitution Avenue, Washington 25, D. C. There are two chapters of general discussion detailing specific information and general tests and methods; four
chapters describing in detail testing procedures of the four most widely used virus vaccines for poultry; and four chapters detailing ways and means of detecting contamination of various sorts in the vaccines. Price is $3.00 per copy.

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EPIDEMIOLOGY AND CONTROL OF A SALMONELLA DUBLIN FOOD INFECTION OUTBREAK IN MAN

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MARY B. DALE, M.D., DR. P.H., Chief, Division of Acute Communicable Diseases, Los Angeles County Health Department

Epidemiology and Control of a Salmonella dublin Food Infection Outbreak in Man

The purpose of this paper is to report an outbreak of Salmonella food infection in humans. This outbreak occurred among the users of milk from a certified raw milk dairy and is considered to have originated from the milk cows on the premises.

Review of Literature:

It is quite clearly defined in the literature that contamination of milk can result in some if the cows in a herd are carriers and shedders of Salmonella organisms. S. dublin has been the type most frequently incriminated in cattle. Buxton (2) describes cases in England where raw milk was contaminated by fecal material from cows which were shedding S. dublin. Outbreaks in humans resulted. Another case, wherein the source of infection was a cow with Salmonella mastitis, is also described. Field (3) in a series of articles published in the British Veterinary Journal (1948) describes in detail bovine salmonellosis outbreaks in England. Rankin and Slavin (4) report Salmonella enteriditis variety dublin infection in a cow resulting in an outbreak of human gastroenteritis. Grunsell and Osborne (5) report on Salmonella dublin infection in adult cattle. The World Health Expert Committee on Zoonoses also discusses the incidence and importance of salmonellosis in the report of their 1958 meeting.

Salmonella dublin has been incriminated as causing enteric infections in calves in the Los Angeles area for several years (6, 7, 8). Approximately 150 such cases have been diagnosed in California according to the records of the Bureau of Livestock Disease Control, California Department of Agriculture. In 1954, Brennan and Laskey (9) also reported on the infection in calves in the area.

History:

The Los Angeles County Health Department reported (1) that cases of Salmonella dublin infection occurred late in October and early November, 1958, among users of certified raw milk from a dairy in the Los Angeles area. On November 19, the Health Department received word of a state laboratory report on Salmonella dublin isolation on a bottle washer in the dairy. Onset of clinical illness in this person was October 29, 1958.
On the same day more reports were received from the State Public Health Laboratory indicating *S. dublin* infection in two individuals who were users of the certified raw milk. In the days that followed, eight additional confirmed cases of *Salmonella dublin* infection were received. Each case had used certified raw milk from the dairy except one, a baby of seven weeks of age who was in the Los Angeles County General Hospital with *Salmonella dublin* meningitis. This baby had been given raw carrot juice purchased from the dairy, which was added to his bottles of milk twice daily.

These 11 laboratory confirmed cases had a range of onset dates from October 29 to November 2, except the baby whose onset of meningitis was November 21. The distribution of the 11 confirmed cases was—one case on October 29, 1958; two cases on October 31, 1958; five cases on November 1, 1958; two cases on November 2, 1958; one case on November 21, 1958.

An additional group of 36 clinical cases of acute diarrhea was possibly related to the use of certified raw milk. Their onsets were from October 30 to November 7, 1958.

17 cases were from the general population with onset dates from October 31, 1958 to November 4, 1958.

8 cases were from the eighty (80) dairy employees and were located as a result of a questionnaire and stool examination survey made in the dairy plant. Onsets October 30, 1958 to November 7, 1958.

11 cases were from four households out of thirty-four (34) households of persons who took certified raw milk. A small sample of the dairy milk route was used to obtain these cases. Their onsets were from October 31, 1958 to November 4, 1958.

The dairy in question is a modern well operated up-to-date plant. It operates under the regulations of the Los Angeles County Health Department and is certified by the Medical Milk Commission.

Three hundred ninety-eight (398) cows are kept on the dairy which is a typical Southern California dry lot operation. The barns and corrals are maintained in excellent sanitary condition. There is no pasture. Replacement animals are purchased from various sources in California and other states. No calves are raised on the premises. All are removed from the dairy on the day they are born.

The records of the Los Angeles County Livestock Department revealed no reports of sick animals or disease outbreaks on the dairy. The veterinarian representing the Medical Milk Commission who makes the required routine inspections also had no record of disease outbreaks among the cattle.

**Epidemiological Investigation:**

It was immediately desirable to determine the source of the *Salmonella dublin* infection. To do so it was necessary to locate all possible cases. The Los Angeles County Health Department checked all laboratory reports of *S. dublin* infection in the area. Histories and stool samples were obtained from the dairy employees and a portion of customers on the dairy's milk
route were questioned. The results of this survey have been outlined previously.

The bottle washer who had a laboratory confirmed report of *S. dublin* with onset of illness on October 29 was first incriminated as a possible source of the infection. However, it seemed that the scope of the outbreak was out of proportion to the amount of contamination that a bottle washer could cause.

It was surmised that since practically all employees in the dairy plant drank the raw milk, it was possible that the bottle washer and the eight others who had diarrhea were victims of contaminated milk.

Further, the laboratory records of daily tests of the milk revealed that the cultures on the milk bottled on October 28, 1958 showed "coliforms too numerous to count." All other laboratory counts for the month were considered to be within safe limits.

Although a great number of milk samples, both individual and pooled, were checked, *Salmonella dublin* was never isolated from the milk.

The raw carrot juice, also considered as a potential source, was purchased in bulk by the dairy and bottled in the milk bottling machine on the premises. Several samples taken of this product were negative for *Salmonella dublin*. Since distribution and use of this product was limited, the possibility that it was the cause of the outbreak is unlikely.

According to the literature, milk most frequently becomes contaminated via the feces of shedder cows.

With this information in mind, it was decided to examine the entire dairy herd in an attempt to identify shedders of *Salmonella dublin* if any were present.

Dr. P. R. Edwards (10) stated that serological methods of locating carrier or shedder animals were generally not dependable. Buxton (2) reports that "in cattle, sheep, goats and pigs the agglutination test is of value provided the interpretation of results is related to the average herd or flock titers in the district and to the age of the animal. The majority of adult cattle have serum agglutinins to *S. dublin.*" No herd surveys for *Salmonella* using the agglutination test have been conducted in this area, thus average herd titers had not been established.

At present the cost accurate method of locating shedder cows is by bacteriological examination of individual fecal specimens.

The collection and processing of such a large number of fecal samples is a considerable task. In all approximately 1,600 rectals were made to secure them.

On December 5 and 6, veterinarians and livestock sanitation men from the Los Angeles County Livestock Department and dairy sanitarians from the Los Angeles County Health Department collected fecal specimens from all the dairy cattle. Cows were identified by eartag number. Precautions were exercised to prevent possible cow to cow infection or contamination of one fecal sample by another.

A total of three hundred ninety-eight (398) specimens were obtained. These were processed by the laboratory of the Livestock Department and suspect Salmonella cultures sent to the Los Angeles County General Hospital
EPIDEMIOLOGY AND CONTROL

for group identification. Species identification was made by the laboratory of the California State Department of Public Health in Berkeley and by the Laboratory of the United States Public Health Service, Chamblee, Georgia.

The results were as follows:

Cow No. 343 — *S. dublin*
Cow No. 390 — “ “
Cow No. 394 — “ “
Cow No. 365 — *S. cubana*

The remainder of the herd was negative for Salmonella. Thus, the presence of *Salmonella dublin* shedder animals in the herd was established.

Procedures Leading to Control:

The Los Angeles County Health Officer required that all milk distributed from this dairy must be pasteurized until such time as the milk was considered safe.

Subsequent conferences held between the Health Officer, Medical Milk Commission, and veterinarians from the Livestock Department resulted in agreement on the procedures for an attempt to eliminate the carrier animals from the herd.

It was decided that a series of fecal examinations be conducted on the cows in the dairy. Accepted procedures for detection of Salmonella carriers for human food handlers (three fecal examinations at two week intervals) were considered to be satisfactory. The owner was anxious to cooperate since he was very desirous of resuming the sale of certified raw milk.

The three *Salmonella dublin* shedders identified earlier were isolated from the remainder of the herd and quarantined.

The first sampling in the series was begun on January 5, 1959. It was necessary to reduce the number of samples to be processed through the laboratory. Therefore, individual fecal samples from 10 cows were placed directly into one bottle of enrichment media. Each composite cow sample thus represented 10 cows. Three hundred eighty-seven (387) cows were represented in this sampling (eight cows checked previously had been sent to slaughter and the three *S. dublin* shedders were isolated from the herd).

The results of this examination were as follows:

Composite cow sample No. 3 — *S. kentucky*
“ “ No. 37 — *S. cubana*
“ “ No. 35 — *S. group B*

*No Salmonella dublin* was found.

The second sampling in the series was conducted on January 19, 1959 and January 20, 1959. No Salmonellas were found.

The third and final sampling in the series was conducted on February 3, 1959. In an effort to allay possible criticism, individual as well as pooled fecal samples were examined. All the three hundred and eighty (380) individual specimens (seven more cows had gone to slaughter) and the thirty-nine (39) composite samples were negative for Salmonellas.
The three cows which had been identified as shedders on the first test were sampled at routine intervals.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cow Number</th>
<th>Date</th>
<th>Cow Number</th>
<th>Date</th>
<th>Cow Number</th>
</tr>
</thead>
</table>

These three cows were subsequently sent to slaughter. Bile specimens were obtained at the meat packing plant and bacteriological examinations were conducted with the following results:

Cow No. 343 — S. dublin (pure culture)
Cow No. 390 — S. dublin " "
Cow No. 394 — Negative

A. Buxton in a personal communication with the authors stated that in his experience a cow may be regarded as an active carrier if Salmonella dublin is isolated from its feces on three successive occasions when the samples have been taken at intervals of 10 to 14 days. An animal is considered not infected if three successive samples are negative. Thus, we designated cows numbers 343 and 390 as carriers, while cow number 394 was not infected. Culture of the bile specimens certainly supports Doctor Buxton’s observation.

In an effort to determine the source of infection in the dairy cows and to gain further information, many specimens, such as feed, milk from the shedder cows, and feces from calves born to the shedder cows were examined.

Dec. 16, 1958 — Cow No. 394 had come fresh
    Milk specimen — Negative for S. dublin
    Feces from calf No. 394 — Negative for S. dublin

Dec. 18, 1958 — Feed sample check
    Dry lot feed — Negative for S. dublin
    Feeders in milk barn — Negative for S. dublin
    Open feed-east side — " " "
    Open feed-west side — " " "

Jan. 13, 1959 — All shedder cows now calved
    Calves allowed to nurse shedder cows.
    Feces from calf No. 343 — Negative for S. dublin
    " " " No. 390 — " " "
    " " " No. 394 — " " "

It is interesting to note that although the calves were kept in the same enclosure as the cows and allowed to suckle them, no evidence of clinical illness was observed in the calves, nor was S. dublin recovered from their feces. Predisposing factors are probably necessary to cause disease in calves in addition to presence of the organism.

Jan. 13, 1959 — Dry cow feed — Negative for S. dublin
    Barn feed — " " "

Inspection of the storage and handling of the feed revealed vermin and bird proof feed enclosures with little or no possibility of contamination.
An open ditch was observed passing through the dry cow lot. It carried surface drainage water from a housing project near the dairy. No sewerage or sewer overflow was involved. The ditch carried only flooding rain water and normal excess from the lawns. This was considered as a possible source of the infection in dairy cattle. Tests of the water revealed no \textit{S. dublin}, however.

In view of the presence of \textit{S. dublin}, particularly in calves in the area, it is most likely that the cows had been infected prior to their entry onto the dairy. The excellent health of the dairy cows on the premises and the good sanitary procedures maintained apparently aided in preventing spread of the infection to other animals.

**Recommendations:**

1. The shedder cows and calves (including cow No. 394 which was considered to be not infected) be sent to immediate slaughter and the meat be heat processed.
2. The drainage condition through the dry cow corral be eliminated.
3. The premises be thoroughly cleaned and disinfected under the supervision of the Los Angeles County Livestock Department.

The owner readily agreed to these requirements.

The County Health Officer then considered that in so far as possible, within known testing procedures, that the dairy was free of \textit{S. dublin} shedders. Thus, no further basis for requiring pasteurization existed. However, responsibility for preventing future occurrences was placed with the Medical Milk Commission. The Commission has recertified the dairy to produce raw milk. It also requires that all cows must have a fecal examination before they are allowed to enter the milking herd from the dry lot. This examination is also required on any newly imported animals.

**SUMMARY**

Highly suggestive circumstantial evidence is presented, indicating that a human outbreak of \textit{Salmonella dublin} diarrhea resulted from contamination of certified raw milk.

Forty-seven cases, 11 of which were laboratory confirmed, occurred chiefly between October 29 and November 4, 1958.

Pasteurization of milk from the dairy was ordered by the Health Officer, pending investigation.

Subsequent sampling of the milk revealed no \textit{Salmonella dublin}. However, fecal samples taken from 398 cows on the dairy revealed \textit{Salmonella dublin} in three animals. Two of these were proven to be active carriers. None were clinically ill nor was there any history of a disease outbreak in the herd.

It is considered likely that a temporary breakdown in hygienic procedure resulted in contamination of the milk by feces from one or more of the shedder animals. This possibly occurred in the milk which was bottled and distributed on October 28, 1958, when an unusually high coliform count was recorded.
All animals shedding *Salmonella dublin* were removed. The remainder of the herd passed three consecutive negative fecal examinations conducted at two week intervals and were then considered not infected.

The Medical Milk Commission allowed the dairy to resume distribution of certified raw milk after it had been cleaned and disinfected under official supervision. As an added precaution, the Commission also now requires that all cows must have a fecal examination before they are allowed to enter the milking herd from dry lot.

At the time of this report, no further cases have been recorded either in man or animal.

**Acknowledgments:**

The authors are indebted to many individuals and groups for active participation, aid, and counselling during this investigation: Roy O. Gilbert, M.D., Carl Lawrence, Ph.D., Walter Wilson and others, in the Los Angeles County Health Department; James Steele, DVM, MPH, R. A. Huffaker, DVM, and others in the United States Public Health Service; A. Buxton, Ph.D., M.R.C. V.S., University of Liverpool; Karl F. Meyer, DVM, MD, University of California, San Francisco; R. V. Jessup, DVM; J. E. Stuart, DVM, and M. D. Moys, DVM, California State Department of Agriculture.

We wish to express our special gratitude to the members of the staff of the Los Angeles County Livestock Department who worked so diligently on this project: C. A. Delli Quadri, DVM, W. L. Rottman, DVM, R. W. McIntyre, DVM, W. H. House, DVM, M. A. McElroy, DVM, Rebello Robusto, James Pecaro, R. L. Phillips and L. F. Meier.

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PUBLIC HEALTH SIGNIFICANCE OF DRUGS IN FOOD

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United States Food and Drug Administration

Washington, D. C.

With the ever-increasing demand for a more abundant and economic food supply there is a tendency to use chemical substances to produce meat quicker and keep it longer. This means that animals are being treated with drugs for the prevention of disease and fed or injected with hormones or hormone-like substances to increase weight gains and feed conversion. Poultry and fish are being dipped in antibiotic solutions to extend their shelf life. Because of these developments, it has become increasingly necessary to protect the food supply for both man and animals from adulteration with toxic or deleterious ingredients.

The average consumer does not have the ability, qualifications, or facilities to investigate the safety of drugs or to evaluate the safety of products from medicated animals. Therefore, he must rely on the integrity of the drug and chemical manufacturers, of the users of the drugs, and the concern and watchfulness of government agencies.

Historically, governmental protection of the consumer dates back to at least the year 300 B.C. when an Indian law prohibited the adulteration of grains, scents, and medicine. Although there were earlier laws to control special items, such as the Assize of Bread in 1202, the first comprehensive English legislation, the Adulteration of Food and Drink Act, was enacted in 1860. In this country, the first attempt to regulate domestic products was made by the individual states. With an increasing proportion of food and drugs being shipped in interstate commerce, it became apparent that federal legislation was needed. Public response to the disclosure of some of the practices in the unregulated food and drug industries stimulated congressional action, which resulted in the Food and Drugs Act of 1906.

Administrative experience and judicial interpretation soon disclosed gaps in the protection afforded by the 1906 law. After a long legislative history, a new act was adopted, called the Food, Drug, and Cosmetic Act of 1938. This act, after introductory chapters defining the terms used and listing general prohibitions and enforcement methods, deals successively with food, drugs, devices, and cosmetics. The term “food” is defined to mean, in part, “articles used for food and drink for man or other animals * * * and Articles used for components of any such articles.” Section 406(a) of the 1938 act at that time read, “Any poisonous or deleterious substance added to any food, except where such a substance is required in the production thereof, or cannot be avoided by good manufacturing practice, shall be deemed to be unsafe for purposes for application of clauses (2) of section 402(a).”
This clause then classed a food to be adulterated "if it bears or contains any added poisonous or added deleterious substance which is unsafe within the meaning of section 406."

In 1954 Congress passed special legislation, referred to as the Miller Amendment for pesticides chemical residues, and substituted for the "required in production," the test of agricultural usefulness.

Persons who wish to promote a pesticide chemical secure evidence about its toxicity to animals, the amount required for the particular purpose, and the amount that will remain on the food after its use. These facts are submitted along with others to the Food and Drug Administration with a request to set a formal tolerance for safe residues of the chemical on specific raw agricultural products. Simultaneously the Department of Agriculture is asked to certify that the chemical is useful. When the Food and Drug Administration has the certificate of usefulness, it determines what amount of the chemical may be consumed daily for a lifetime without any harm. Using all this data, a safe legal tolerance is set as the level to be permitted. No amount of the particular chemical may be permitted in excess of that which meets the needs of agriculture. If the chemical is too toxic to remain on food in any amount, the tolerance is set at zero. If the chemical is relatively harmless so that any foreseeable use of it will not be a hazard to the public health, it may be exempted from the requirement of a tolerance.

When a tolerance is set for a pesticide chemical this means that: (1) crops in interstate commerce should bear no more than the tolerance level of residue (2) residues within the tolerance are safe (3) the chemical is useful and (4) when used properly the chemical will not leave residues above the safe level.

The Food and Drug Administration will not establish a tolerance for a toxic compound in forage if the feeding of this forage gives residues in animal tissues unless the petitioner has presented evidence to show that the residues in milk and meat are safe and the Food and Drug Administration simultaneously grants tolerance for the residues in milk and meats. Thus far no tolerance for a chemical residue in milk has been granted. Tolerances for pesticidal residues have been established on a number of agricultural crops. They cover residues resulting both from the application of pesticides on growing crops and from post-harvest application. Tolerances for pesticidal residues apply only to the raw agricultural products. It is our obligation to see that established tolerances are observed and that unauthorized residues are not present.

The next point of interest is the effect, if any, of processing upon the pesticidal level in a processed article, for example, a commercial animal feed. The amount of pesticide cannot exceed that which is permitted on the raw product unless authorized by the Food Additives Amendment, which will be discussed later.

The problem of residues which might occur in the edible tissues of animals as a result of the drugs which the animals have received, is assuming increasing proportions. At the present time it may be conservatively estimated that approximately 7 to 9 million tons of medicated feeds are manufactured in
this country each year. This does not take into account the tons of drugs used in drinking water or those administered by other means. For example, it is estimated that 75 tons of antibiotics are used annually for intramammary infusion for the prevention and treatment of bovine mastitis. The following are a few of the drugs used in feeds for the prevention and treatment of animal diseases: Antibiotics, nitrofuran, organic arsenicals, sulfonamides, various coccidiostats, tranquilizers, and phenothiazine.

The addition of a drug to animal feed, in essence, makes a drug out of the feed to which it is added. These drugs come under the drug provisions of the Federal Food, Drug, and Cosmetic Act, which defines the term “drug” to mean, in part, any “articles intended for the diagnosis, cure, mitigation, treatment, or prevention of disease in man or animal” and “articles (other than food) intended to affect the structure or function of the body of man or other animals.” In addition, the Act defines the term “new drug” to mean, in part, “Any drug the composition of which is such that such drug is not generally recognized among experts qualified by scientific training and experience to evaluate the safety of drugs as safe for use under the conditions prescribed, recommended, or suggested in the labeling thereof.”

Before the Food and Drug Administration permits a new drug application to become effective, evidence relating to the safety of such a drug along with adequate data of research using the drug is required.

A number of years ago a new drug application was submitted for the use of thiouracil in animal feed to promote fattening of swine. After several preliminary conferences with the manufacturer, the Food and Drug Administration had to request additional data to satisfy itself that treated animals would be safe for human consumption. This experience led to the publication in the Federal Register of December 4, 1948 of a notice to manufacturers, packers, and distributors of veterinary preparations and animal feeds. This notice, in part, stated, “In considering a new drug application for a product intended to effect physiological changes in farm animals, the Department of Health, Education, and Welfare will regard absence of satisfactory evidence showing that the meat or other food obtained from animals fed the drug is entirely free of any poisonous or deleterious ingredient resulting therefrom at the time of marketing as grounds for refusal to make the application effective.”

Another section of the law which has had a profound impact on the food supply is that which deals with the class of products which are generally referred to as “certifiable antibiotics.” These provisions of the law and the regulations issued under them outline what must be done in order to market a product that is in full compliance with the federal law. Regulations covering medicated feeds with certifiable antibiotics (chlortetracycline, penicillin, streptomycin, bacitracin, chloramphenicol, and derivatives of these compounds) are for the most part summed up in regulation 146.26. This regulation comprises about 30 pages and is probably the legal basis for the present-day marketing of approximately 75 percent of all medicated feeds. Briefly, these regulations provide for the use of the certifiable antibiotics in medicated feeds by themselves or in combination with other drugs. To be covered under these regulations and not be in violation of the law, the feed must
conform with these regulations which define the tag claims, feeding directions, potency, and in some cases, contain requirements of the new drug provisions of the law when a new drug is being used with the certifiable antibiotic in the same feed.

Nothing could be clearer than the intent of Congress to have this act cover feed and medication for livestock and other animals, as well as food and drugs for human beings. The statute prohibits the adulteration of food (including feed, by definition) and drugs and requires among other things adequate directions for use and warnings against misuse to appear prominently on the labels for such articles.

To those who wonder why the Food and Drug Administration in the enforcement of the Federal Food, Drug, and Cosmetic Act is playing a more important role in the regulation of the feed industry, the national importance of medicated feeds should be emphasized. As mentioned before, this class of products presents numerous and highly complex scientific and technical problems. Over and above all, however, looms one paramount consideration—protection of the public health. Medicated feeds cannot be viewed simply in terms of their economic or medical effect on food animals and the food they produce. All of us must be primarily concerned with the question of whether the food for man obtained from treated animals could possibly have any adverse effect on the human consumer.

Until the passage of the new food additives bill, which amended the 1938 act, untested or inadequately tested chemicals could enter the food supply. When a manufacturer chose to use new chemicals and decided that he did not care to conduct adequate toxicity tests, the burden of determining whether the new chemical should be in food then fell upon someone else, the Food and Drug Administration. We did not, nor do not, have the facilities to conduct independent tests on even a significant fraction of all the new compounds being proposed for food use.

In March 1959, the provisions of the Food Additives Amendment to the federal law, enacted in September 1958, became operative. Substances covered by the Food Additives Amendment are those additives not generally recognized by competent experts as having been adequately shown to be safe under the conditions of their intended use. The amendment covers substances that are added intentionally to food as well as those that may reasonably be expected to become a component of food. Pesticide chemical residues in processed foods must be considered under this new law. Substances used in food or feeds that are generally recognized as safe or which have been established as safe through scientific procedures are exempt from the provisions of the amendment. Also exempt are substances that have had prior approval by the government for use in foods or feeds under the Federal Food, Drug, and Cosmetic Act, the Meat Inspection Act, and Poultry Products Inspection Act.

Briefly, the new amendment makes the following requirements with respect to additives:

(1) The available data must establish that the proposed use of the additive under the conditions to be specified in the regulation will be safe.
(2) No additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal.

(3) The additive must not, under the proposed conditions of use, promote deception or otherwise cause a food to come misbranded or adulterated under any provisions of the Act.

(4) Where it is necessary to affix a tolerance limitation in order to assure that the proposed use is safe, the tolerance set must not be greater than for the amount necessary to achieve the intended physical or other technical effect, and none will be permitted if the proposed use fails to accomplish this effect.

Once a regulation authorizing the use of an additive has been published, any person who wishes to use it may do so in conformity with the terms of the regulation without further clearance of approval from the Food and Drug Administration.

It is recognized that the application of the amendment to animal feed has given rise to much concern and speculation. This has been particularly true with respect to those feeds which contain estrogenic-like substances and arsenicals. The Federal Register of May 30, 1959, contained, in part, the following statements:

“(a) Section 409(c)(3)(A) of the Federal Food, Drug and Cosmetic Act provides that ‘no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additive, to induce cancer in man or animal.’ This applies whether such additive becomes a component of food directly or indirectly. A veterinary drug may become a food additive through addition to the animal’s feed or drinking water, whether or not residues of the drug become a component of human food derived from the animal. A veterinary drug may become a food additive, regardless of the route of administration if, as a result of its use, residues of the drug or its conversion products become a component of human food derived from the animal.

“(b) The following policy will be observed by the Food and Drug Administration in considering new-drug applications and supplements to new-drug applications for veterinary drugs and in considering proposed amendments to the antibiotic-drug regulations for veterinary drugs which fall within the meaning of section 409(c)(3)(A) of the act:

“(1) Such a substance may be approved for animal use as a drug pursuant to the provisions of sections 505 and 507 of the act, provided that any residues of the drug or any conversion product falling within the meaning of section 409(c)(3)(A) of the act does not become a component of human food derived from the animal, and provided further that the substance is not administered as a component of food, including the drinking water supply.
"(2) No such substance can be permitted as a component of animal feed or in the drinking water supply, whether or not residues of the drugs or their conversion products become a component of meat, milk, or eggs of the treated animals.

"(3) Authorization pursuant to section 505 or 507 of the act for the use of such a substance cannot be granted if the substance or any conversion product falling within the meaning of section 409(c) (3) (A) becomes a component of human food derived from the treated animals.

"(4) No supplement nor any amendment for any purpose to an existing authorization under sections 505 and 507 for the use of such a substance can be approved if the original application or regulation which it seeks to supplement or amend is not itself one that could be made effective under the policy announced in this statement.

"(e) The Food and Drug Administration recognizes that some actions taken under sections 505 and 507 of the act prior to the enactment of section 409(c) (3) (A) might not be possible under the policy stated in this section. The status of these existing authorizations will be reviewed, and if it is concluded that revocation of new-drug applications or amendment of antibiotic regulations will be required in the proper administration of the Federal Food, Drug, and Cosmetic Act appropriate action will be taken."

Under the law any substance to be added to animal feed that is not itself generally recognized as safe must be shown to be safe for the animal under the intended conditions of use. Where this cannot be demonstrated, authorization to use the additive will be denied forthwith. Where safety to the animal can be established then the petitioner must demonstrate either that the edible products of the animal—meat, milk, or eggs—are free of any residues of the substance and its degradation products, or where such residues are found, that they will be safe for consumption by man or other animals, as the case may be. That, in a nutshell, is the requirement the Food Additives Amendment imposes on animal feed.

It is of interest that the first additive cleared under this procedure was for an antioxidant (Santoquin brand of ethoxyquin) used in animal feed. It is of further interest to note that the Food and Drug Administration, in clearing that additive for use, established tolerances for its residues in edible beef and poultry products of animals consuming the treated feeds as well as for levels in the feed itself.

In summary, the Food, Drug, and Cosmetic Act is a consumer protection law. It forbids interstate commerce in adulterated or misbranded food, drugs, devices, and cosmetics. Foods and drugs for animals are subject to the same provisions of the law as are the foods and drugs for humans. Specifically these include the new drug provisions, the certificate antibiotics provisions, the pesticide chemicals amendment of the act, and the most recent, the food additive provisions of the law.
All of us who are interested in livestock production must primarily be concerned with the question of whether the food for man obtained from treated animals can possibly have any adverse effect on the human consumer. If there are any questions of safety to be resolved, they must be always resolved in favor of public health protection.

From a public health viewpoint there are two questions that must be asked concerning the administration of drugs to food-producing animals. First, is there any possible harm to the consumer of products from treated animals?, and second, what benefits occur from their use? We think that the benefits, such as the availability of more and better foods at less cost to producer and consumer and the control of many animal diseases transmissible to man, must not result in any possible hazard to mankind. When properly used according to label directions, we feel certain that drugs in current use do not endanger the public health. However, we must always be on the alert for the indications of misuse.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH

R. J. Schroeder, Chairman, Los Angeles, California; A. L. Brueckner, College Park, Maryland; G. H. Good, Cheyenne, Wyoming; H. J. Rollins, Raleigh, North Carolina; O. Sussman, Princeton, New Jersey; R. H. Huffaker, Los Angeles, California.

As indicated by its name the business of this Committee is public health. This being the case it is fitting that the theme of this report be closer cooperation between veterinarians and public health officials. Prompt and efficient exchange of pertinent information is necessary for each to do the best possible job. As an example; public health officials should be interested in the occurrence of encephalomyelitis in horses and birds as an indication of virus activity in the community and a forewarning of possible human disease. Veterinarians in turn should look for animal cases when leptospirosis or brucellosis is diagnosed in man. For mutual benefit joint epidemiological investigations and information exchanges are indicated in many of the diseases now diagnosed, recorded, filed, and forgotten by both groups.

Health of Dairy Cattle as Relating to Public Health

In the 62nd Annual Report it was recommended that studies of a manual outlining methods for use in health inspections and examinations of dairy cattle be continued. The Committee has made further study of this subject and of the problems presented; the required frequency of examinations, the difficulty in diagnosing certain diseases by clinical examination alone, determining exactly what condition should disqualify a cow as a milk cow and obtaining cooperation of the dairyman. A program which removes cows from production without offering something in the way of cure or correction will not be enthusiastically received by the dairy industry. It is suggested that in addition to the annual physical examination now required in some areas a program of continuous herd supervision should also be carried out.

This might be called a "continuous check" (1) method whereby the veterinary practitioner who cared for the herd would increase his services during routine visits. His approach would be through a herd health program and should be well received by the dairyman. Certain signs are warnings of trouble or emerging trouble in the herd. They do not necessarily cause clinical disease which requires a veterinarian's attention. Such things as increased turnover in milk cows, herd milk production decreases, increased mastitis rates, poor conception rates, high milk bacteria counts, as well as abortions and increased calf mortality all point to trouble. A card or form should be used on which this and similar information would be entered as a herd health record. Corrective procedures would be taken by the owner and veterinarian as they are indicated.
The basic problem is to milk healthy cows in order to produce good milk. To obtain healthy cows one must have a healthy herd. We may be attempting to solve the problem a difficult way by setting up rigid criteria as to what constitutes a healthy cow as far as milk production is concerned. Why not work for a healthy herd and acquire the healthy cows as a consequence?

The Salmonella Problem

The problem of the salmonella contamination of foods, both for animals and man, and the salmonellosis which results has increased in the past few years. Contamination of protein concentrates for animal feeds has resulted in infections in animals (2). Dogs have been repeatedly shown to excrete salmonella serotypes in their feces. Many of these same serotypes have also been isolated from human infections. Milk-borne infections of *S. dublin* have been reported from Europe and in 1958 from Los Angeles (3).

Increased movement of animal foodstuffs in world commerce further spreads the organisms. Specific salmonella serotypes are reported from new areas for the first time each year.

This Committee recommends that further study be encouraged on the role played in transmission of salmonellosis by animal feeds and that the effect of commercial methods of sterilization of these foodstuffs be investigated.

Tuberculosis

It has been estimated that there are 50,000,000 tuberculin skin test positive people in the United States (4). It is from this population that most of the human cases will develop as time passes. These individuals in whom tuberculosis becomes active are capable of infecting other people and a variety of animals including cattle.

With this in mind, the public health authorities in Minnesota are operating a joint tuberculosis eradication program with the State Department of Agriculture. When a human case of tuberculosis is diagnosed the Department of Agriculture is informed. The Department then locates animal contacts and tuberculin tests them. Conversely, when cattle react to the tuberculin test the health department is notified and persons having contact with the cattle are skin tested and X-rayed if it is indicated. Both public health and agriculture are benefiting from the program.

We recommend that similar programs be adopted in other areas.

Q. Fever

New and better laboratory techniques and increased interest have demonstrated Q fever in many areas and at rates higher than seen previously. Since the disease is of clinical importance to man but so far as is known is not of clinical or economic importance to animals it has received little interest from veterinarians and other animal regulatory officials.

Because of the public health importance of Q fever and its repeated isolation from cattle and sheep throughout the United States this Committee wishes to encourage further research, especially in the area of immunization and to determine if it coexists as part of other infectious processes of animals.
Brucellosis

The public health problem of brucellosis has not been discussed too often to bear repetition. There is a continued decrease in the human attack rate as a result of the efforts made in livestock brucellosis control. The 1958 report from Minnesota by Held and others graphically illustrates the close relationship between brucellosis in man and cattle. As the infection rate in cattle increased or decreased there was a corresponding change in the rate of human brucellosis infection (5).

Staphylococcic Phage Typing

There has been a great deal of interest in phage typing of staphylococci found in milk since typing of the so-called "hospital staphylococcus" has become commonplace. In this regard it must be pointed out that phage typing is an epidemiological tool and not a decisive "fingerprint" which will always point out what micrococcus was responsible for the infection. Work by several research groups has shown that many of the staphylococci found in milk cannot be lysed by the phages used to type staphylococci isolated from man. More phages must be isolated if phage typing is to be of assistance in determining where a certain staphylococcus strain infecting cattle originated and how it was spread.

Typing can be expected to yield information regarding staphylococcus in udders that were infected from a human source, i.e., boils on the milkers' hands, staphylococcal rhinitis of milkers, and so forth. It will be of great interest to determine what phage types occupy udders without causing disease and what types are usually pathogenic. Studies of this type are now underway in several laboratories.

Antibiotics in Fluid Milk

The fourth nationwide survey of antibiotics in fluid milk has been conducted and reported by The Food and Drug Administration, United States Department of Health, Education and Welfare (6).

The results of the four surveys are as follows:

<table>
<thead>
<tr>
<th>Year of Survey</th>
<th>No. of Samples</th>
<th>Samples that Contained Penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>1954</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>1955</td>
<td>474</td>
<td>55</td>
</tr>
<tr>
<td>1956</td>
<td>1,706</td>
<td>101</td>
</tr>
<tr>
<td>1959</td>
<td>1,170</td>
<td>43</td>
</tr>
</tbody>
</table>

The 1959 survey shows a significant drop in the percentage compared to the 1955 and 1956 figures. This reduction is attributed to:

(1) Education of the dairy farmer
(2) The requirement that all drug containers have a warning label which instructs the dairy farmer to discard milk for 72 hours after the last treatment, and,
(3) Limitation of the quantity of penicillin permitted in mastitis preparations for intramammary use to 100,000 units per dose.
It was further the opinion of Jester, et al., that many of the antibiotic positive samples found in the fourth survey would have been negative if they had been collected after having been diluted with antibiotic free milk at the processors. In the first three surveys this was the case. In the fourth the samples were collected from tank trucks or directly from individual producers.

The investigators state that the program has not completely solved the problem of penicillin-contaminated milk.

The Public Health Committee fears that if contaminated milk continues to be detected it is possible that the use of penicillin for intramammary infusion will be prohibited by law. Such a ruling would eliminate a very important aid in the fight against costly mastitis. We urge everyone concerned to intensify their efforts, particularly in education, in hope that drastic legislation will not be necessary.

**Chemical Feed Additives, Systemic Insecticides, and Livestock Sprays**

The use of chemical feed additives, systemic insecticides, and livestock sprays must be very carefully investigated by the Association. The utilization of such products has been of immense value to the livestock industry. However, each product should be subjected to severe scrutiny before it is approved for widespread use. The recent catastrophe involving the use of a chemical weed killer in the cranberry industry is an excellent example. This chemical which was used on only a portion of the crop was considered to be a potential danger to human health. The resultant publicity dealt a severe blow to the entire industry. It is possible that a similar incident could happen to the livestock industry. We recommend that the Association utilize its every resource to prevent such a calamity.

**Recommendations**

2. Utilize the “Herd Health Approach” rather than individual animal examination in the production of wholesome milk.
3. Further study is encouraged on the role played in transmission of salmonellosis by animal feeds and commercial methods of sterilization of these foodstuffs.
4. Closer cooperation between public health authorities and animal regulation officials engaged in tuberculosis control must be encouraged for mutual benefit.
5. Research on Q fever should be continued with particular reference to immunization and the part Q fever may play in other infectious processes of animals.
6. Results of phage typing of staphylococcus mastitis microorganisms now underway in several laboratories should be closely observed by the Public Health Committee.
7. Intensify the educational programs directed toward the elimination of penicillin-contamination in milk.
8. The Association should utilize every resource to safeguard the public and the livestock industry in the use of chemical feed additives, systemic insecticides and livestock sprays.

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THE ROLE OF THE SPOTTED SKUNK IN RABIES

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The study of diseases of wildlife has many compensations. It leads to the study of nature in general, because the animal and vegetable kingdoms are interdependent. However, as one scientist remarked: "It was a dubious pleasure to study the spotted skunk, because it was like enjoying a cigar in a powder magazine." Having suffered the retaliatory effects of this skunk on numerous occasions while collecting them in nature, and when observing them in the laboratory, I agree with this scientist, but I still continue to study this very interesting animal.

Man can learn much from the animal world and I want to comment on what we can learn from the skunk. I will introduce the subject by quoting from a poem by Pope:

"Go, from the creatures thy instructions take;
Learn from the birds what food the thickets yield;
Learn from the beasts, the physic of the field;
The arts of building from the bee receive;
Learn from the mole to plow, the worm to weave;
Learn of the little Nautilus to sail
Spread the thin oar, and catch the driving gale."

Well, what can we learn from the skunk. There is an analogy in the science of weaponry of which we hear so much these days. The striped skunk must at one time have been a timid and furtive creature, fearful of destruction by the larger, swifter, carnivorous animals, but it developed a weapon that is now respected by all animals and against which there is no sure defense. The striped skunk is not afraid of any creature, even the wildcat will retreat when this animal threatens. The skunk does not suffer from the use of its weapon and hence does not hesitate to use it. I am sure you recognize the analogy to the military defense weapons now available to man.

From the spotted skunk we can learn further about the protective devices of wild animals. This weasel-like skunk has a variety of other names, such as "polecat," "civet," "civet cat," "hydrophoby cat," and "phoby cat." It has developed a coat of fur which, with its erratic pattern of black and white,

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makes this nocturnal animal invisible at night, even if it is in the direct moonlight, as long as it remains still. Thus it relies on camouflage as one means of protection. It has the same gaseous weapon as the striped skunk and it has developed an interesting threatening gesture whereby it stands on its forepaws and rears up and aims directly before firing. In addition, this animal appears to have a built-in biological weapon, that is, rabies virus, and this is the main subject of our discussion.

Before enumerating the various kinds of evidence we have for this hypothesis, I will comment on parasitism in general and about rabies as we know it around the world. The study of diseases of wildlife shows us that the parasitic microbial agents which have become established in a natural host do not tend to produce obvious disease or epidemics of disease in this host, under the conditions of the long term history of the species. Changes produced in the hosts' ecology by natural events or by man may result in the production of disease, for example, overcrowding, changes in the water table so that the ground that is usually dry becomes damp or vice versa, destruction of competing animals by disease or by man, etc. However, when a parasite strays into aberrant hosts it often produces devastating epidemics which reduce the affected species to a point where the remaining individual families are widely separated. Canine rabies is an example of aberrant parasitism of this type. The innate capacity of the virus to produce encephalitis is the means for its survival in this type of host. It must make the animal mad and so increase the natural tendency to bite and it must also invade and multiply in the salivary glands in order to continue the cycle of infection. In wildlife hosts such as the fox, coyote, wolf and jackal, the disease disappears eventually, but migrating epidemics may continue for many years before the affected species becomes reduced to a point where the chain of infection is broken. We do know that there are intervals of many years during which there is no evidence of rabies in the wild canine hosts in certain regions of the world, and then the disease recurs. Under such circumstances we must consider the possible sources of the virus in nature. The capacity of rabies virus to multiply in a variety of organs such as the salivary gland, kidney, and breast tissues makes it possible for the virus to have cycles of infection not associated with encephalitis. The fact that rabies virus can be cultivated in the tubular epithelium of the kidney tissue of hamsters shows us that the virus does not need nervous tissue in order to multiply and survive (1). There is considerable similarity between rabies virus and the poliomyelitis viruses of man and certain animals, and we must consider the possibility that rabies virus may, under certain circumstances, survive as an intestinal tract infection. The carnivorous animals form the obvious hosts of rabies, and it may be that the virus strays into aberrant hosts, using the intestinal tract as a portal of entry. It is to be expected that the virus will experience some difficulty in becoming established in the wild canine host because it does not always reach the salivary glands, and it may so overwhelm the nervous system that the affected animal is not stimulated to bite or is unable to do so.
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that the infection in this host is not associated with encephalitis except under most unusual circumstances. The occurrence of the virus in the breast tissue suggests to us that the infection may be propagated from mother to young via the milk. The route of infection of the virus could be via the respiratory tract or by way of the intestinal tract. One can consider the possibility of infection of the young via the saliva when the mother animal bites off the umbilical cord. This seems improbable because of the lack of nervous tissue in the umbilical cord. It has not been possible to demonstrate a viremia in laboratory animals infected with the spotted skunk strain of rabies virus and this makes it unlikely that the virus will reach the embryo via the blood stream.

There is a remarkable consistency of infection in the young when the virus is transmitted by the mother animal, much higher than we can achieve with experimental inoculation, other than via the intracerebral route. For example, on April 16, 1957, three baby striped skunks were found by a man near Vallejo, Solano County, California. Two of the skunks were sold as pets and one was kept by the man that found them. On July 10th, one of these skunks died and was found to have rabies. On July 26th another one of the skunks died and it too was positive for rabies. On July 29th the third skunk died and it was positive for rabies. One can surmise that the baby skunks were found because they left their homes in search of food after the mother had died of rabies. The incubation periods of 116, 132 and 135 days are unusually long but their length may be related to the route of infection. I want to remark here that had these skunks been vaccinated with the Flury strain live virus vaccine the vaccine would have been blamed for producing the death of the animals. Several young skunks have developed rabies after vaccination with the live virus dog vaccine and their deaths were attributed to the vaccine.

The cycle of activity of rabies virus may be postulated as follows: When the population of weasels and skunks is moderate, we can expect that the population of wild canines such as the coyote and fox will be at a comparable level. The precipitating factor in the spread of the rabies virus to aberrant hosts would be an over-population of carnivores. This would result in crowding of individual families and opportunity for clashes as they protect their hunting grounds. The increase in the population of carnivores depends on an abundance of small mammals. As the population of small mammals declines, the carnivores will compete more intensively and the likelihood of clashes will increase. Here is where the Mustelids have the advantage if they can produce a disease in their competitors. The outbreak of rabies in coyotes in California and Oregon in 1910-1915 appears to have exterminated the coyotes in certain regions and they have not yet become abundant in any area of these states. We do know that the population of small mammals has reached unusually high levels in California during the past seven years, and we know also that in some areas the small mammal population has decreased abruptly. Spotted skunks and weasels will consume from four to six mice a day in the laboratory, in terms of a year over 1,000 mice per animal. There must have been a problem as regards food for the spotted
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skunks because in some urban communities where these animals have never before been seen they have been killed when searching for food in and under houses.

The observations which I have reported to you show that the spotted skunk is involved in the long term ecology of rabies virus in western United States. Although there is not so much evidence to incriminate the weasel, this animal may be even more important than the spotted skunk for the maintenance of the virus.

REFERENCES

The role of the spotted skunk in rabies

The natural history of rabies indicates that the permanent hosts of the virus are to be found in the families Mustelidae and Viverridae. In the far north there have been several self-limited epidemics of rabies in arctic wolves and foxes. There has been no evidence of the importation of the virus into this region and the disease has not been observed during the inter-epidemic periods. In the arctic regions the weasel or ermine, Mustela erminea, has been found infected with rabies on at least one occasion (2). I believe that this animal is the reservoir of rabies virus in the arctic region. The mongooses of the subfamily, Herpestinae, and the civets of the subfamily, Viverrinae, appear to be the source of rabies virus in Asia. In South Africa, the polecat, Ictonyx striatus, the African weasel, Poecilogale albinucha, and the civet, Civettictis civetta, are occasionally found to be infected with rabies virus but the yellow mongoose, Cynictis penicillata, is the principal source of human rabies. The weasels and related Mustelids are found in the arctic regions, the Temperate Zones, the tropics and the far south. Whether these animals form the main source of rabies virus in nature remains to be proved but they are one of the known wildlife hosts and yet do not suffer epidemics of rabies. Furthermore, they are the one host which is found in all countries in which the disease occurs.

The spotted skunk, Spilogale putorius, has been the source of isolated cases of rabies in man and domestic animals in the absence of other recognized sources of the virus. One can say that this host is one of the reservoir hosts of rabies virus in the western half of the United States. One weasel was found to be infected with rabies in Riverside County, California, in 1951 and another in Los Angeles County in 1952. There were six proved cases of skunk rabies in California in 1951 and nine in 1952. Three of the nine were in Los Angeles County. The epidemic of rabies in striped skunks which began in California in 1954 has continued to the present time and has involved more than 30 counties. A total of 868 skunks were found positive for rabies by laboratory examination during the period 1954-1958 inclusive, but less than 10 of these were spotted skunks and there were no known cases of rabies in weasels during this period. The population of striped skunks has been high, and one can but surmise that the epidemic originated from spotted skunks or weasels and that the precipitating factor was an over-population of mustelid carnivores.

The study of the life history of the spotted skunk indicates that the long term habitat of this animal has been Mexico and southwestern United States and that it moved north and east since the last glacial period and possibly only in the last few hundred years (3). The variation in color pattern of white and black spots and stripes are so infinite that no two of these animals have been found with precisely the same pattern. Little is known of the ecology of this animal. The pygmy species, Spilogale pygmaea, which lives in western Mexico, is known by only four specimens. This is the most primitive of the skunks. The races of spotted skunks in Mexico and Yucatan are smaller and have more white pelage as compared with the races in the northern part of the United States which are larger and blacker (4). The deserts of the southwest and the arid grasslands of the great plains are the
preferred habitat of the spotted skunk. This animal can live for an indefinite period without water. It can be kept alive for months on a diet of small mammals which it consumes completely, including bones and fur. This nocturnal mammal is agile and can climb trees and steep rocky surfaces. It makes its home in burrows on rocky slopes, under the roots of trees or under wood piles. It is seldom recognized by man unless it is run over by an automobile and found lying on the road. It was unknown in many parts of the United States until the pelt became popular as a fur and then it was possible to obtain an abundance of pelts by trapping. For example, a total of 2,114,535 Spilogale pelts were sold on the fur market during the period 1919-1921 (5). The abrupt cessation of the trade in skunk and fox furs may have had something to do with the current large scale epidemic of rabies in wildlife.

The earliest reference to skunk rabies in North America is that of Duhaut-Cilly who, when he visited Cape San Lucas, Lower California, in 1826, noted that the people there told him that the spotted skunk sometimes entered houses at night and bit people and gave them hydrophobia (6). Nelson was in Cape San Lucas on January 1, 1906, when a skunk entered a house and bit a child. There is no record of what happened to this child as he left the region soon afterwards. Rev. Hovey was one of the first to write about the relationship of skunk bite to rabies in western United States (7). During the period 1873-1875 at least 40 persons, such as cowboys, soldiers and hunters, died of rabies in Kansas after they had been bitten by skunks while sleeping on the ground (8, 9). Coues quotes a letter from Dr. J. G. Tidball of California, telling about a disease like hydrophobia which had been observed among miners, following the bite of the Spilogale skunk during the gold-rush period (10). Ten cases of rabies from skunk bite were reported in Arizona during the period 1907-1910 (11). From local traditions one can learn of many other cases of rabies from skunk bite in western United States. The human cases of rabies were, for the most part, the result of bites by the spotted skunk and this is why this animal is known as the “phoby cat” in the southwest.

The recent general outbreak of wildlife rabies in the United States began in 1940 in the southeastern states with the gray fox as the epidemic host (12). A few skunks were found positive for rabies during the first few years of this epidemic but it was the fox which was the means for the dispersion of the disease throughout the eastern half of the country. In 1953, rabies was identified in insectivorous bats in Florida (13). Since that time there have been many isolated cases of bat rabies in various parts of the United States. The evidence available at the present time suggests that bats are occasionally infected with rabies by predatory carnivores, such as the spotted skunk or weasel, as a part of the general involvement of a large variety of mammals in the current epidemic of rabies in wildlife. When rabies is introduced among colonial bats such as the Mexican freetail bat, it may occur in epidemic proportions in this host in the same manner as that observed in vampire bats in Mexico and South America but we will have to wait until the present epidemic of wildlife rabies subsides before we will be able to
THE ROLE OF THE SPOTTED SKUNK IN RABIES

determine whether the disease can persist in colonial insectivorous bats in the United States. I do not believe that it will.

Beginning in 1952, skunk rabies appeared in many widely separated regions of the United States with no evidence of any other wildlife host of the disease. By 1955 a total of 24 states had reported skunk rabies and the disease had reached epidemic proportions in striped skunks in California, Iowa, Minnesota, South Dakota, Texas and Wisconsin (14, 15). The spotted skunk and weasel are abundant in each of these states but only a few cases of rabies have been identified in these animals. For example, on June 24, 1952, a weasel entered a farmyard in Mower County, Minnesota, and bit a dog. The weasel did not appear vicious but could not be chased away. It was killed by the farmer but the brain was not examined for rabies virus. On June 12, 1955, a farmer in Freeborn County, Minnesota, came out of his house early in the afternoon and saw a weasel running about the yard chasing a dog. The farmer killed the weasel and sent it to the State Health Department where it was found positive for rabies by demonstration of Negri bodies in the brain. On July 7, 1958, a woman on a farm in Yellow Medicine County, Minnesota, observed a weasel fighting the domestic cats in the yard in daytime. The weasel was killed but the brain was not examined for rabies. On October 3, 1958, a farmer in Jackson County, Minnesota, was in the chicken house debeaking chickens when a weasel entered the chicken house and began attacking the chickens. The farmer killed the weasel but the animal was not examined for rabies (16). The proportion of spotted skunks to striped skunks found positive for rabies in Minnesota is similar to that in California; for example, in 1956 there were 106 striped skunks and five spotted skunks found positive for rabies. It is of interest to note that a mink, Mustela vison, was also found positive for rabies that year (17).

It is evident that the spotted skunk is not the only source of rabies virus in the United States, but there are two lines of study which show that this animal is an important wildlife source of rabies in western United States. One is the investigation of the isolated cases of rabies that do occur in spotted skunks. The other is the study of the virus obtained from this animal. I will describe a human case of rabies that occurred in California in 1954 as the result of the bite of a spotted skunk. On October 22nd, a 58-year-old woman was on a hunting trip with her husband at Sugarloaf Mountain, Tulare County, California. She was bitten on the little finger of the right hand and on the left elbow by a spotted skunk while sleeping in a sleeping bag on the ground. The skunk was killed by the woman’s husband. Though there was no history of rabies in this region for many years, the hunters were familiar with the tradition that the spotted skunk could transmit the disease and at the advice of the local forest ranger the skunk was sent to the State Health Department for examination. The immediate microscopic examination of the animal brain was negative for Negri bodies and though she was advised to take the rabies vaccine treatment anyway, the woman decided not to do so because there was no definite evidence that the biting animal had rabies. The skunk brain was tested for rabies virus by the mouse inoculation test, and on November 18th the mice developed symptoms of
rabies, and Negri bodies were found in the brains of the mice that developed paralysis. The rabies vaccine treatment was begun on November 22nd but the woman developed symptoms of rabies on November 30th and died on December 3, 1954. Rabies virus was isolated from the brain of this woman. An interesting feature of this case was the long incubation period of the virus infection in mice inoculated with the virus from the skunk and the woman’s brain. There is no record of other cases of skunk rabies in the area where this woman was bitten.

The character of the spotted skunk strain of rabies virus has been studied, using a specimen obtained from a spotted skunk in 1957. On October 14th this skunk invaded a farmyard near the Sacramento River, at Orland, Glenn County, California, and bit a dog and attacked a man in daytime. The animal was killed and though no person was bitten by the animal, its actions indicated that it was rabid. Knowing the possible importance of this type of case, Dr. E. M. Smith called the laboratory and asked if we were interested in having the skunk. We said that we wanted the entire animal sent in for study. It was a young female spotted skunk and, in addition to the tests of the brain and salivary glands, the kidney and breast tissues were taken for animal inoculation studies. These were washed carefully in saline to remove the blood in the tissue. The animal was not lactating but yet the breast tissue was positive for rabies virus. The kidney was also positive for rabies virus. The virus was reisolated from both the breast and kidney specimens after storage in sealed ampoules in the carbon dioxide dry ice chest. The kidney tissue was used to inoculate four young spotted skunks. Each of these received 0.03 ml of the 10 percent kidney tissue suspension in each thigh muscle. The titer of the virus in the kidney tissue suspension was >0.015 ml $\times 10^{-3}$ IC LD$_{50}$ in infant mice. One of the inoculated spotted skunks died of rabies at 32 days and the other at 52 days after inoculation. This strain of rabies virus has been studied because of its unique character, especially the long incubation period in mice inoculated intracerebrally and the unusual symptomatology of the disease in the laboratory mouse. Negri bodies are not found in adult mice inoculated intracerebrally with this strain of virus but they occur in mice receiving the brain tissue of mice from the first intracerebral passage. This indicates a lack of prior experience of the virus with the brain tissue. The virus has been maintained without intracerebral passage, using hamster kidney tissue culture, chick embryo inoculation by the yolk sac route and intramuscular passage in spotted skunks, using the submaxillary salivary glands as a source of virus. The virus does not always invade the salivary glands in spotted skunks that die of the disease but when present it may reach a titer of >0.015 ml $\times 10^{-7}$ IC LD$_{50}$ in mice. This virus strain can also be isolated from the pancreas in the spotted skunks which were infected by intramuscular injection with the virus derived from the submaxillary salivary glands. This suggests an affinity for entodermal tissues.

In general, one can say that the character of the spotted skunk strain of rabies virus is different from that of strains obtained from dogs during epidemics of dog rabies. It has certain characteristics which lead one to surmise that the virus is not spread among spotted skunks by biting and
REPORT OF THE COMMITTEE ON RABIES

E. S. TIERKEL, Atlanta, Georgia, Chairman; M. J. CEROSALETTI, Albany, New York; H. R. Cox, Pearl River, New York; J. W. MANN, Atlanta, Georgia; L. E. STARR, Atlanta, Georgia; R. L. WEST, St. Paul, Minnesota.

RABIES INCIDENCE AND TRENDS — CALENDAR YEAR 1958

Animal Rabies—National totals for calendar year 1958, add up to 4,814 laboratory confirmed cases which is comparable to the 1957 total of 4,802 (Agricultural Research Service, United States Department of Agriculture, Communicable Disease Center, United States Public Health Service) (Figure I). The geographic distribution of the 1958 cases is also similar to the 1957 picture. The midwestern states, particularly Minnesota, Wisconsin and Iowa again experienced extensive rabies epizootics among skunks which account for the majority of confirmed cases in these three states as well as in North and South Dakota. The increased incidence in Indiana; from 120 in 1957 to 338 in 1958 is largely due to a canine epizootic in Marion County—Indianapolis. Louisiana reports a significant reduction from 179 cases in 1957 to 31 in 1958. Mississippi likewise has had a sharp decline and so far in 1959 has not reported a case. New England and the upper Rocky Mountain States remain rabies free, except for three cases in Colorado, in coyotes and skunks.

For the second year on record, wildlife rabies cases outnumbered canine cases. This is especially significant in light of the more than doubling of dog and cat populations since 1945. The first year during which positive diagnoses in wildlife exceeded those in dogs was 1957, during which 1,758 dogs and 1,942 wild animals were confirmed rabid. There were 1,096 additional cases among cattle, horses, sheep, swine, cats, goats and man which bring the grand total to 4,802 for 1957. The margin is wider for 1958 with 1,643 confirmed canine cases and 2,075 for sylvatic fauna. The grand total for 1958 was 4,814. Rabies virus was isolated from a bat in Nebraska during 1958, which made this the 19th state which has reported confirmed rabies in one or more bats since the first report in Florida in 1953.

In 1945 the dog population was estimated at about 10,000,000 and in 1958 at more than 26,000,000. If dog rabies had continued at the 1945 level of 8,505, more than two and a half times as many cases or 21,000 would have occurred in 1958, when in reality there were only the 1,643 confirmed canine cases. The reduction of the high rate of canine rabies in the past decade in face of the over-all increase of disease in wild animals is significant tribute to the effectiveness of canine rabies immunizing practices.

National Rabies Picture—First Nine Months—1959

The incidence of animal rabies during the first nine months of 1959 is 2,851 cases, according to the weekly telegraphic reports to NOVS, United States Public Health Service. It will be noted that there has been a decrease
of 754 cases from the same period in 1958, representing a 20 percent decline in total reported cases (Table I). Substantial drops in rabies incidence occurred in Ohio, Indiana, Wisconsin, Minnesota, Virginia, West Virginia, South Carolina, Georgia, Kentucky and California. The biggest increase was in New York State which reported 276 cases for the first nine months of 1959 as compared to 182 cases for the same period during 1958. This increase was due to an epizootic of fox rabies in seven counties of previously rabies-free western New York State and represents the worst outbreak in the state since 1946.

Human Rabies—1958

Six human rabies deaths were reported during 1958 (See Table II). Two of the six fatalities contracted the infection from stray dogs. One case was caused by fox bite, one by bat bite and one by skunk bite. In one case, the vector is unknown. Three of the cases received no specific antirabies treatment. In two cases, 14 daily doses of antirabies vaccine were administered and the patients succumbed to rabies after very short incubation periods. In the case reported from California the patient received antirabies serum as well as 14 doses of antirabies vaccine begun four days after the bite. This is the first completely documented case of human rabies caused by bat bite. The patient was bitten by a silver-haired bat (Lasionycteris noctivagans) which was positive for rabies by both microscopic and the animal inoculation tests. The bitten woman developed rabies two months after exposure. (Two other human deaths were previously attributed to bat exposures, one in 1951 and the other 1956, in neither of these episodes was the biting bat examined.)

Human Rabies Deaths—1959 to Date

Table III gives the human rabies deaths reported to the United States Public Health Service from January 1st to November 27, 1959. Perhaps the most striking fact shown here is that two of the five cases were attributed to exposure by bats. In fact, in less than a 12-month period (11/4/58-9/3/59), three cases of human rabies in the United States were attributed to bats.

It will be noted that in only one of the five human rabies cases reported thus far this year was post exposure immuno-prophylactic treatment given; this was in a 10-year-old boy in Atlanta, Georgia bitten by a confirmed rabid dog. The dog in the case was the last rabid animal reported in Atlanta this year. In two of the five cases reported, the victims were under 15 years of age. The incubation periods of the three known cases were 22, 40, and 86 days respectively. The case in Birmingham, Alabama had a rather long (15 days) clinical course.

Highlights of Year’s Progress

During the year, wildlife investigations carried out by the Communicable Disease Center showed that foxes were more susceptible than skunks to challenge with a fox rabies virus isolate from southeastern United States. It was also shown that in skunks which do succumb to the same isolate, the
incubation period and the clinical course of illness is longer, the present virus positive salivas were greater and the virus titers of saliva were higher than in counterpart groups of infected foxes.

Studies have been carried out in two phases of the fluorescent antibody technique, one in routine brain specimens sent to the laboratory from the field for diagnosis and the other in brain and salivary gland specimens from experimentally infected and thoroughly studied rabid animals. In the first study, the FA test was in complete agreement (100 percent) with the mouse inoculation test on a total of 825 fresh or frozen brain specimens. The FA test detected all of the 70 positives included in the group. In contrast, in only 66 of 70 positives (94.3 percent) were Negri bodies demonstrable. In the other study, in 44 brain specimens and 33 salivary gland specimens, one brain and three salivary glands were positive by the FA test which was negative by the mouse test. This test is a valuable adjunct to the armamentarium of the rabies diagnostician.

The Animal Quarantine and Inspection Branch of the Agricultural Research Service revised its minimum requirements for production of chicken embryo rabies vaccine as of November 6, 1959. The new revised standards require that guinea pig potency retests be carried out on vaccine samples from field distribution points at five months after original release of the product. Vaccine lots which fail to pass this retest will be recalled from market channels. Those which pass the retest will be retested again about seven months later, just before expiration date.

The Communicable Disease Center, United States Public Health Service established its third regional rabies station during the year. The Midwestern Rabies Investigations Station at Poynette, Wisconsin was formally opened on September 23rd and 24th, 1959 by CDC's Chief, Assistant Surgeon General Robert J. Anderson. Research in wildlife rabies was featured at the scientific sessions which were held during the two day meeting. This station will carry out wildlife rabies investigations principally in skunks and will serve the rabies control authorities of a ten-state region from the Dakotas east to Ohio in all phases of rabies control problems. Similar stations were established previously in the Southwest (Las Cruces, New Mexico) where emphasis is put on bat rabies investigations and in the Southeast (Montgomery, Alabama) where investigations in fox rabies is the primary mission.

**Bat Rabies**

The number of bat rabies cases reported in the United States since the first reported case is 359. Table IV gives the distribution of these cases by state, type of bat and date of report. As pointed out in the section on incidence and trends, Nebraska was the only new state to report one or more cases of bat rabies during 1958 making it the nineteenth state to do so.

During 1959, five new states were added to the list making a total of 24 states which have reported one or more cases of rabies in bats since the first report from Florida in 1953. These new states were West Virginia, Connecticut, Virginia, Illinois and Maryland in that order.
Table V gives the distribution of bat rabies cases reported by type of bat. It will be noted that there has been four species of tree-living or solitary bats and 20 known species of colonial bats involved thus far.

Thus far, five human rabies deaths have been attributed to exposure by bats. In four of these cases, bat transmission was based on epidemiological and anamnestic evidence and in one, the biting bat was available for diagnosis and the case was thoroughly investigated by epidemiological, clinical and laboratory methods.

**TABLE I**

*Rabies in Animals*  
First Nine Months—1958-1959  
(NOVS—United States Public Health Service)

<table>
<thead>
<tr>
<th>States</th>
<th>1958 (First 9 Months)</th>
<th>1959 (First 9 Months)</th>
<th>States</th>
<th>1958 (First 9 Months)</th>
<th>1959 (First 9 Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New England</strong></td>
<td></td>
<td></td>
<td><strong>East South Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td>0</td>
<td>Kentucky</td>
<td>402</td>
<td>180</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>0</td>
<td>0</td>
<td>Tennessee</td>
<td>103</td>
<td>102</td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td>0</td>
<td>Alabama</td>
<td>155</td>
<td>170</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0</td>
<td>0</td>
<td>Mississippi</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Middle Atlantic</strong></td>
<td></td>
<td></td>
<td><strong>West South Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>182</td>
<td>276</td>
<td>Arkansas</td>
<td>98</td>
<td>201</td>
</tr>
<tr>
<td>New Jersey</td>
<td>0</td>
<td>0</td>
<td>Louisiana</td>
<td>22</td>
<td>43</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>50</td>
<td>20</td>
<td>Oklahoma</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td><strong>East North Central</strong></td>
<td></td>
<td></td>
<td>Texas</td>
<td>315</td>
<td>349</td>
</tr>
<tr>
<td>Ohio</td>
<td>158</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>187</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>27</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michigan</td>
<td>32</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>183</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>West North Central</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>374</td>
<td>211</td>
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<tr>
<td>Iowa</td>
<td>180</td>
<td>149</td>
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<td>Missouri</td>
<td>124</td>
<td>139</td>
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<tr>
<td>North Dakota</td>
<td>51</td>
<td>47</td>
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<tr>
<td>South Dakota</td>
<td>41</td>
<td>90</td>
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<td></td>
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</tr>
<tr>
<td>Nebraska</td>
<td>26</td>
<td>44</td>
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<tr>
<td>Kansas</td>
<td>0</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>South Atlantic</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Delaware</td>
<td>0</td>
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<td></td>
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</tr>
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<td>Maryland</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>District of Columbia</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>223</td>
<td>127</td>
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<td></td>
</tr>
<tr>
<td>West Virginia</td>
<td>105</td>
<td>56</td>
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</tr>
<tr>
<td>North Carolina</td>
<td>24</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Carolina</td>
<td>100</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgia</td>
<td>173</td>
<td>115</td>
<td></td>
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<tr>
<td>Florida</td>
<td>48</td>
<td>52</td>
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</tr>
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</table>

Totals 3,605 2,851
### TABLE II

**Human Rabies Deaths—1958**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lawrence County, Ohio</td>
<td>2/1/58</td>
<td>4</td>
<td>F</td>
<td>Bite on forehead</td>
<td>36 days</td>
<td>3 days</td>
<td>None</td>
<td>Stray dog</td>
</tr>
<tr>
<td>2. Clarendon County, South Carolina</td>
<td>3/4/58</td>
<td>60</td>
<td>F</td>
<td>Multiple bites on fingers</td>
<td>22 days</td>
<td>3 days</td>
<td>Local cleansing, 14 doses vaccine</td>
<td>Fox, confirmed rabid</td>
</tr>
<tr>
<td>3. Athens, Ohio</td>
<td>9/20/58</td>
<td>10</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>4 days</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>4. South Dakota</td>
<td>10/6/58</td>
<td>2</td>
<td>M</td>
<td>Bite</td>
<td>16 days</td>
<td>10 days</td>
<td>14 doses vaccine</td>
<td>Skunk, confirmed rabid</td>
</tr>
<tr>
<td>5. Atlanta, Georgia</td>
<td>10/8/58</td>
<td>55</td>
<td>M</td>
<td>Severe bite, nose</td>
<td>14 days</td>
<td>11 days</td>
<td>None</td>
<td>Stray dog</td>
</tr>
<tr>
<td>6. Butte County, California</td>
<td>11/4/58</td>
<td>53</td>
<td>F</td>
<td>2 bites, fingers</td>
<td>57 days</td>
<td>8 days</td>
<td>Serum systemically, 14 doses vaccine, 4 days after bite</td>
<td>Bat, confirmed rabid</td>
</tr>
<tr>
<td>Locality</td>
<td>Date Died</td>
<td>Age</td>
<td>Sex</td>
<td>Nature of Exposure</td>
<td>Incubation Period</td>
<td>Length of Illness</td>
<td>Treatment</td>
<td>Biting Animal</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td>--------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-----------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>1. Los Angeles County, California</td>
<td>6/3/59</td>
<td>54</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>9 days</td>
<td>None</td>
<td>Unknown—Probably bats in Texas caves</td>
</tr>
<tr>
<td>2. Birmingham, Jefferson County, Alabama</td>
<td>6/8/59</td>
<td>30</td>
<td>F</td>
<td>Bite on finger</td>
<td>Approx. 86 days</td>
<td>15 days</td>
<td>None</td>
<td>Neighbor's dog</td>
</tr>
<tr>
<td>3. Sullivan County, Indiana</td>
<td>7/7/59</td>
<td>4</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>8 days</td>
<td>None</td>
<td>Unknown—Probably dog</td>
</tr>
<tr>
<td>4. Richland County, Wisconsin</td>
<td>9/3/59</td>
<td>44</td>
<td>M</td>
<td>Bite on ear lobe</td>
<td>22 days</td>
<td>6 days</td>
<td>None</td>
<td>Bat—not examined</td>
</tr>
<tr>
<td>5. Atlanta, Fulton County, Georgia</td>
<td>10/3/59</td>
<td>10</td>
<td>M</td>
<td>Bite on left arm above elbow</td>
<td>40 days</td>
<td>3 days</td>
<td>21 doses of vaccine</td>
<td>Dog—confirmed rabid</td>
</tr>
</tbody>
</table>

**TABLE III**

*U. S. Human Rabies Deaths*

*January 1—November 27, 1959*
<table>
<thead>
<tr>
<th>States</th>
<th>Species</th>
<th>No.</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>Lasius seminolus</td>
<td>2</td>
<td>Aug.-Sept. 1956</td>
</tr>
<tr>
<td>Arizona</td>
<td>Eptesicus fuscus</td>
<td>3</td>
<td>May 1957</td>
</tr>
<tr>
<td></td>
<td>Antrozus pallidus</td>
<td>4</td>
<td>June 1957</td>
</tr>
<tr>
<td></td>
<td>Myotis velifer</td>
<td>5</td>
<td>May 1958</td>
</tr>
<tr>
<td></td>
<td>Lasius cinereus</td>
<td>2</td>
<td>May 1957</td>
</tr>
<tr>
<td></td>
<td>Tadarida brasiliensis mexicana</td>
<td>4</td>
<td>August 1957</td>
</tr>
<tr>
<td></td>
<td>Pipistrellus hesperus</td>
<td>1</td>
<td>August 1959</td>
</tr>
<tr>
<td>California</td>
<td>Tadarida brasiliensis mexicana</td>
<td>9</td>
<td>July 1954</td>
</tr>
<tr>
<td></td>
<td>Myotis californicus</td>
<td>1</td>
<td>June 1955</td>
</tr>
<tr>
<td></td>
<td>Lasius seminolus</td>
<td>4</td>
<td>September 1956</td>
</tr>
<tr>
<td></td>
<td>Lasionycteris noctivagans</td>
<td>1</td>
<td>September 1958</td>
</tr>
<tr>
<td></td>
<td>Myotis evotis</td>
<td>1</td>
<td>September 1957</td>
</tr>
<tr>
<td></td>
<td>Macrotrus californicus</td>
<td>1</td>
<td>December 1958</td>
</tr>
<tr>
<td></td>
<td>Myotis sp.</td>
<td>1</td>
<td>August 1956</td>
</tr>
<tr>
<td></td>
<td>Isolation not yet identified</td>
<td>1</td>
<td>July 1959</td>
</tr>
<tr>
<td></td>
<td>(Marin County)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado</td>
<td>Unknown</td>
<td>1</td>
<td>August 1959</td>
</tr>
<tr>
<td>Connecticut</td>
<td>Lasionycteris noctivagans</td>
<td>1</td>
<td>October 1959</td>
</tr>
<tr>
<td>Florida</td>
<td>Myotis australiparius</td>
<td>1</td>
<td>1953</td>
</tr>
<tr>
<td></td>
<td>Myotis grisescens</td>
<td>1</td>
<td>February 1955</td>
</tr>
<tr>
<td></td>
<td>Pipistrellus subflavus</td>
<td>3</td>
<td>September 1955</td>
</tr>
<tr>
<td></td>
<td>Lasius borealis</td>
<td>3</td>
<td>October 1954</td>
</tr>
<tr>
<td></td>
<td>Lasius seminolus</td>
<td>10</td>
<td>July 1953</td>
</tr>
<tr>
<td></td>
<td>Dasypterus floridanus</td>
<td>20</td>
<td>June 1953</td>
</tr>
<tr>
<td></td>
<td>Tadarida brasiliensis cynocephala</td>
<td>8</td>
<td>April 1955</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>2</td>
<td>May 1959</td>
</tr>
<tr>
<td>Georgia</td>
<td>Lasius seminolus</td>
<td>3</td>
<td>1956</td>
</tr>
<tr>
<td></td>
<td>Lasius borealis</td>
<td>4</td>
<td>1956</td>
</tr>
<tr>
<td>Illinois</td>
<td>Myotis lucifugus</td>
<td>1</td>
<td>October 1959</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Tadarida brasiliensis cynocephala</td>
<td>1</td>
<td>1955</td>
</tr>
<tr>
<td></td>
<td>(1 pool pos. containing 4-5 bats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>Lasius cinereus</td>
<td>1</td>
<td>November 1959</td>
</tr>
<tr>
<td>Michigan</td>
<td>Eptesicus fuscus</td>
<td>2</td>
<td>November 1956-1957</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Lasius seminolus</td>
<td>1</td>
<td>August 1956</td>
</tr>
<tr>
<td>Montana</td>
<td>Eptesicus fuscus pallidus</td>
<td>3</td>
<td>August 1954, 1955, 1957</td>
</tr>
<tr>
<td></td>
<td>Myotis californicus</td>
<td>13</td>
<td>July 1955, 1957-1958</td>
</tr>
<tr>
<td></td>
<td>Myotis evotis evotis</td>
<td>8</td>
<td>Oct. 1956, July 1958, September 1959</td>
</tr>
<tr>
<td></td>
<td>Myotis evotis chrysonotus</td>
<td>1</td>
<td>July 1957</td>
</tr>
<tr>
<td></td>
<td>Myotis lucifugus</td>
<td>1</td>
<td>May 1959</td>
</tr>
<tr>
<td></td>
<td>Myotis volans interior</td>
<td>7</td>
<td>Aug. 1957-Jan 1958</td>
</tr>
<tr>
<td>States</td>
<td>Species</td>
<td>No.</td>
<td>Date</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-----</td>
<td>-------------------</td>
</tr>
<tr>
<td>Nebraska</td>
<td>Lasiurus cinereus cinereus</td>
<td>1</td>
<td>August 1955</td>
</tr>
<tr>
<td></td>
<td>Isolutions not yet identified (1 collected in Hamilton—1957.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 obtained from a pool of the brains of 2 bats near French-town in Missoula County—May 1959</td>
<td>2</td>
<td>1957, May 1959</td>
</tr>
<tr>
<td></td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>August 1959</td>
</tr>
<tr>
<td></td>
<td>Lasionycteris noctivagans</td>
<td>1</td>
<td>September 1959</td>
</tr>
<tr>
<td></td>
<td>Lasionycteris</td>
<td>1</td>
<td>August 1959</td>
</tr>
<tr>
<td></td>
<td>Myotis yumanensis</td>
<td>1</td>
<td>June 1958</td>
</tr>
<tr>
<td></td>
<td>Corynorhinus townsendii</td>
<td>7</td>
<td>June 1958</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico</td>
<td>Myotis lucifugus</td>
<td>2</td>
<td>August 1958, 1959</td>
</tr>
<tr>
<td></td>
<td>Lasiurus borealis</td>
<td>1</td>
<td>August 1959</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico</td>
<td>Tadarida brasiliensis mexicana</td>
<td>98</td>
<td>1955-1957</td>
</tr>
<tr>
<td></td>
<td>Tadarida molossa</td>
<td>3</td>
<td>Aug.-Sept. 1958</td>
</tr>
<tr>
<td></td>
<td>Lasiurus cinereus</td>
<td>1</td>
<td>November 1955</td>
</tr>
<tr>
<td></td>
<td>Lasiurus cinereus</td>
<td>1</td>
<td>August 1957</td>
</tr>
<tr>
<td></td>
<td>Lasiurus cinereus</td>
<td>2</td>
<td>June, August 1958</td>
</tr>
<tr>
<td>New York</td>
<td>Eptesicus fuscus</td>
<td>3</td>
<td>August 1956, 1958</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1</td>
<td>June 1959</td>
</tr>
<tr>
<td>Ohio</td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>pool</td>
</tr>
<tr>
<td></td>
<td>Myotis lucifugus</td>
<td>1</td>
<td>pool</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oklahoma</td>
<td>Tadarida brasiliensis mexicana (pool of 4)</td>
<td>1</td>
<td>August 1956</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panama Canal</td>
<td>Lasiurus cinereus</td>
<td>1</td>
<td>June 1957</td>
</tr>
<tr>
<td>Zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(not included in U. S. total)</td>
<td>3</td>
<td>June 1959</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Lasiurus cinereus</td>
<td>1</td>
<td>September 1953</td>
</tr>
<tr>
<td></td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>June 1958</td>
</tr>
<tr>
<td>Texas</td>
<td>Tadarida brasiliensis mexicana</td>
<td>58</td>
<td>November 1953</td>
</tr>
<tr>
<td></td>
<td>Antrozous pallidus</td>
<td>1</td>
<td>1955</td>
</tr>
<tr>
<td></td>
<td>Myotis velifer</td>
<td>1</td>
<td>June 1958</td>
</tr>
<tr>
<td></td>
<td>Lasiurus cinereus</td>
<td>1</td>
<td>September 1958</td>
</tr>
<tr>
<td></td>
<td>Dasypterus floridanus</td>
<td>2</td>
<td>August 1958</td>
</tr>
<tr>
<td></td>
<td>Lasiurus borealis</td>
<td>10</td>
<td>July 1954</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1</td>
<td>July 1959</td>
</tr>
<tr>
<td>Utah</td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>July 1956</td>
</tr>
<tr>
<td>Virginia</td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>September 1959</td>
</tr>
<tr>
<td>West Virginia</td>
<td>Unknown</td>
<td>2</td>
<td>July-August 1959</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>Myotis lucifugus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eptesicus fuscus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Total of 6 positive for 1957 and 3 positive for 1958—no record as to species)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lasiurus borealis</td>
<td>1</td>
<td>September 1959</td>
</tr>
<tr>
<td></td>
<td>Eptesicus fuscus</td>
<td>1</td>
<td>September 1959</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>359</td>
<td></td>
</tr>
</tbody>
</table>
TABLE V
Species Distribution of Bat Rabies in the U. S.

**Solitary**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lasiurus cinereus</em></td>
<td>16</td>
</tr>
<tr>
<td><em>Lasiurus borealis</em></td>
<td>19</td>
</tr>
<tr>
<td><em>Lasiurus seminolus</em></td>
<td>15</td>
</tr>
<tr>
<td><em>Dasypterus floridanus</em></td>
<td>22</td>
</tr>
</tbody>
</table>

**Colonial**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lasionycteris noctivagans</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis mexicana</em></td>
<td>170</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis cynocephala</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Tadarida molossa</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Pipistrellus subflavus</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Pipistrellus hesperus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Myotis australiriparius</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Myotis lucifugus</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Myotis californicus</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Myotis grisescens</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Myotis velifer</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Myotis volans</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Myotis evotis</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Myotis evotis chrysonotus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Myotis yummanensis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Antrozous pallidus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Eptesicus fuscus</em></td>
<td>20</td>
</tr>
<tr>
<td><em>Macrotus californicus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Corynorhinus townsendii</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Myotis sp.</em></td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
</tr>
</tbody>
</table>

359
Mr. F. G. Buzzell: Next on our program is a symposium on scrapie and Dr. H. Marsh is chairman of the panel and I shall ask him to introduce the members of the panel. Doctor Marsh.

Dr. Hadleigh Marsh: Thank you, Mr. President the members of the panel who will discuss the scrapie problem including both the research work and control and eradication phases. We are very fortunate in having with us the directors of the two research institutions in Great Britain which have done very extensive research on scrapie. These gentlemen have been in the United States for some time and have spoken at several meetings across the country and probably some of you present at these meetings have had an opportunity to hear them.

At this time I would like to introduce the members of the panel as a group before we start on individual talks. They are: Dr. W. S. Gordon, Director of the British Field Station, Compton, England; Dr. John T. Stamp, Director of Moredun Institute, Edinburgh, Scotland; Dr. W. J. Hadlow, Neuropathologist, Compton, England; and Dr. J. L. Hourigan, United States Department of Agriculture, Washington, D. C. We have about one hour available for this discussion and we are going to try to arrange it so that each one of these four gentlemen will have an opportunity to present his material and leave us about 15 or 20 minutes at the end of their presentation for discussion. I am glad to have the honor of welcoming these guests to this, the sixty-third Annual Meeting of the United States Livestock Sanitary Association, particularly since I had the opportunity of visiting these men and enjoying their hospitality in July 1958 in their laboratories. I will ask Doctor Gordon to start the discussion, please.
DOCTOR GORDON: It is a great privilege and pleasure to have the opportunity of speaking to you here in San Francisco. You have given us a very warm reception, and I trust our contributions will be of interest to you.

Scrapie is a most intriguing and baffling disease problem of sheep. It is one which has been the cause of controversy from time to time, but information on its true nature is gradually accumulating. It is an insidious disease characterised by a progressive degenerative disorder of the central nervous system accompanied by clinical signs indicative of a sensation of intense irritation of the skin, tremors and incoordination of gait. Affected animals rarely recover. The disease is inoculable and has been known in Western Europe for over two centuries where it has waxed and waned in different breeds over long periods of years. While the incidence of infection is not usually high the disease can sometimes cause devastating loss in flocks of pure bred sheep, but it is not usually the cause of serious loss in commercial flocks. Scrapie was first diagnosed in the United States in 1947 and it has spread, within one breed, to many states. As a result, the United States Department of Agriculture introduced an eradication programme, in 1953, based on research work carried out in Europe, mainly in Britain.

Apart from important scientific reasons for determining the nature of an apparently unique type of transmissible agent there are important practical reasons for learning more about this disease and its method of spread. Its occurrence has gravely interfered both with national and international sheep trade and it is to the mutual benefit of the United States, Great Britain and other countries in which the disease occurs, or may occur, to press on with its investigation.

(1) Experimental Transmission of the Disease

When first I became associated with the investigation of scrapie, I thought it had the appearance of being a hereditary disease, and was not likely to be inoculable. However, over the course of years, several incidents occurred which convinced me that scrapie was inoculable and that, under certain circumstances, the possibility of spread from affected to healthy animals at pasture could not be excluded.

The sequence of events that led me to hold this view was, firstly, the result of an experiment, started in 1932 by Dr. Russell Greig (1) at Moredun Institute, Edinburgh and which I observed while a member of his staff. In this experiment, Greig exposed a breeding flock of normal sheep to a scrapie contaminated environment for a period of three years. At the end of the three-year period, since no case of scrapie had occurred in the normal flock or their offspring born in 1934 and 1935, the animals were given to a farmer who was prepared to accept them in spite of their history. The flock then
SCRAPIE PANEL

comprised 16 ewes (12 Half-Breds and four Cheviots) and nine female offspring. The male lambs had been castrated and retained at the Institute. Three months after the flock had been transferred to the farmer, one of the Half-Bred ewes developed scrapie, and eventually a total of 10 cases occurred, seven Half-Bred ewes, one Cheviot ewe and two of the female offspring. The first case developed three years and three months, and the last case, five years and two months, after the commencement of the experiment. Apart from field evidence, this was the first experimental warning that the disease could spread by contact.

Secondly, in 1934 at Moredun Institute, I was completing work on the investigation of Louping-ill which showed it to be an encephalomyelitis of sheep caused by a filterable virus transmitted by the tick *Ixodes ricinus*. For four years, 1931-1934, I had carried out field trials to test the efficiency of a vaccine for the prevention of Louping-ill, in which half of the sheep on affected farms were vaccinated and the other half left as controls. Many thousands of sheep were involved in these trials which proved that the vaccine was safe and effective, since it caused no reaction in inoculated sheep, and it reduced the mortality from about 10 percent to about one percent. The vaccine was inoculated subcutaneously, and consisted of a 10 percent suspension in saline of the brain, spinal cord and spleen harvested from sheep which, five days previously, had been experimentally infected with the virus of Louping-ill. The vaccine contained 0.35 percent of formalin which inactivated the virus of Louping-ill.

![Fig. I](image)

*Louping-ill Vaccine 1935*

<table>
<thead>
<tr>
<th>Batch I</th>
<th>Batch II</th>
<th>Batch III</th>
</tr>
</thead>
<tbody>
<tr>
<td>25,000 Doses</td>
<td>18,000 Doses</td>
<td>5,000 Doses</td>
</tr>
</tbody>
</table>

Prepared from 114 sheep; 8 were yearlings born in 1934 from ewes which began to develop scrapie 15 months later in 1936. Some surviving lambs, fellows to the 8 used for vaccine in 1935, developed Scrapie in 1936/7

Scrapie developed in 7 percent of inoculated animals
of 1935 a considerable amount of this vaccine was made for issue on a commercial basis. The various batches of vaccine were blended into three main batches labelled I, II and III, and they were used to inoculate sheep in March and April, 1935. It will be seen from Figure I that the Batch II vaccine contained a factor capable of producing scrapie which was not present in Batches I and III. The first cases were reported 15 months after the vaccine had been applied. Bearing in mind the long interval that had elapsed since vaccination and that the vaccine contained 0.35 percent of formalin and had passed the usual sterility tests, I found it difficult to believe that these cases of scrapie were associated with vaccination. On further enquiry amongst the users of vaccine, it became clear that Batch II was contaminated with an agent that was causing scrapie, and that on farms on which it had been used, scrapie was occurring in varying incidence from a fraction percent to 30 percent on different farms. On tracing the origin of the animals used to make the three batches of vaccine, it was found that of 114 sheep used to make Batch II, eight were castrated male lambs born in 1934 in Doctor Greig’s normal ewe flock which had been maintained in a scrapie environment. Since two lambs, which were fellows to the eight included in the vaccine died of scrapie in 1936 and 1937, it is probable that one or more of the eight lambs included in the vaccine, although quite normal when used were in the incubative stage of a scrapie infection. This convinced me in no uncertain fashion, that scrapie was an inoculable disease.

Thirdly, in 1938, I set up an experiment to confirm that the disease was transmissible (2, 3, 4, 5). A summary of the result is shown in Figure II from which it will be seen that during the first two-year period after the experiment was commenced, scrapie was confined to animals inoculated with tissues from scrapie sheep. In the second two-year period, however, the disease began to appear in sheep inoculated either with saline and broth or normal sheep tissues. Further, it also made its appearance in a non-inoculated control. This confirmed the transmissibility of the disease by inoculation, and again provided the warning that it could spread by contact.

Fourthly, following this work, Dr. D. R. Wilson (6) working at Moredun Institute, confirmed the transmissibility of the disease, that the agent was filterable, that it could be passed indefinitely from group of sheep to group of sheep with cell-free filtrates, and that it could withstand high temperatures and abases from chemical agents that no other animal virus was known to withstand. Doctor Stamp, whom you will hear later, and his staff have confirmed and extended Doctor Wilson’s work.

It may be concluded, therefore, that scrapie is an inoculable disease and that the transmissible agent can be passed in series indefinitely from sheep to sheep with cell-free filtrates prepared from the tissues of infected sheep. This is a characteristic normally associated with the activity of a filtrable virus.

(2) **The Nature of the Transmissible Agent**

The transmissible agent can withstand boiling and exposure to concentrations of chemical agents which inactivate all other known viruses.
Fig. II

Summary of Result of Scrapie Transmission Experiment 1938 to 1942

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Inoculum</th>
<th>Number of Sheep</th>
<th>Incidence of Scrapie</th>
<th>First Case In Time After Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Scrapie Percent</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Saline and Broth, equal parts</td>
<td>60</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Suspending fluid for all tissues</td>
<td>60</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10 percent Normal Sheep Brain, Cord and Spleen</td>
<td>60</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10 percent Scrapie Brain, Cord and Spleen</td>
<td>60</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Supernatant fluid from Centrifuged</td>
<td>60</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Scrapie Brain, Cord and Spleen 3,000 R. P. M.</td>
<td>60</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Gradoch membrane Filtrate of above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supernatant fluid A. P. D. 0.69 u</td>
<td>60</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Scrapie Brain, Cord and Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heated at 60° C. for 30 minutes</td>
<td>60</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Scaprie Brain excluding Medulla 1/5,000,000</td>
<td>10</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50,000</td>
<td>20</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/5</td>
<td>20</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Scaprie Medulla and Cord 1/5,000,000</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50,000</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/5</td>
<td>20</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50</td>
<td>19</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/5</td>
<td>20</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Scaprie Spleen 1/5,000,000</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50,000</td>
<td>19</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/50</td>
<td>19</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&quot; &quot; &quot; &quot; 1/5</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Intracerebral</td>
<td>20 percent Scaprie Brain and Cord</td>
<td>30</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Not inoculated</td>
<td>Controls</td>
<td>30</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>10 percent Scaprie Brain, Cord and Spleen</td>
<td>70 sheep</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

Sheep killed each month after inoculation for histological examination of medulla. Vacuoles were not observed in the nerve cells until clinical signs were manifest at 21 months.
Moreover, the transmissible agent has shown no evidence of producing antibodies despite many attempts by various workers to demonstrate their presence. Further, the lesions of nerve cell degeneration in the brain of affected animals do not resemble those usually associated with a virus infection. The incubation period after inoculation is also peculiar since it is seldom less than four months and may extend to 15 months and upwards. Another baffling and very inconvenient characteristic, from the research point of view, is the wide variation in susceptibility to experimental infection not only between different breeds of sheep but also between families within a breed. This difference in susceptibility has not been shown to be due to the presence of antibodies in resistant animals which suggests that the genetic constitution of the animals concerned is an important factor in determining infection. The variation in susceptibility together with the long incubation period has made progress in research, using sheep as experimental animals, slow and frustrating. The fact that the filterable agent of scrapie has some characteristics that differ from those of known filterable viruses has led to much speculation about its true nature and it is hoped that the work now in progress will ultimately characterise the agent more precisely.

(3) The Natural Method of Spread

The natural method of spread of scrapie is the subject of considerable controversy. In countries where the disease is enzootic, it has shown an ability to spread from one pure breed to another. How such spread occurs is a matter for conjecture. It could be by interbreeding when upgrading flocks or it could be by contact between breeds. Great difficulty has been experienced in determining experimentally whether one or both methods are involved. Present evidence clearly favours the view that the disease passes in families from infected parents to some of their offspring. Nevertheless, numerous authors have provided evidence, largely from field observations, that the disease may spread by contact and Greig's experiment and my own, described above, confirm this view. Under experimental conditions, in which the animals are housed indoors, there has been no recorded instance of spread by contact from diseased to healthy animals. Consequently, it may be concluded that spread within families is probably more readily apparent than spread by contact, but there is a need for more experiments to clarify this point. At Compton we have recently had scrapie develop in a goat that was infected by feeding it with a suspension of brain tissue from an affected animal. The incubation period was 17 months. This animal was in contact with other cases of scrapie in goats, and therefore may have contracted the disease by contact, and not as a result of the dose administered. However, the incubation period of 17 months is within that normally expected following subcutaneous inoculation. Nevertheless, the fact remains that this is the only case of scrapie that we have observed in a goat in which the animal was not inoculated.

If this observation is confirmed—and we are trying to do so both in sheep and goats—it would make spread of the disease through ingestion, or by contact, an acceptable possibility.
(4) Variation of Susceptibility

In Britain there are 36 different registered breeds of sheep, and at Compton samples of 24 of these breeds have been kept as breeding flocks of approximately 30 ewes each. About 40 animals from each of these flocks have been tested for susceptibility to experimental infection with scrapie. This has demonstrated that there is an astonishingly wide difference in susceptibility among the samples of the various breeds tested, ranging from no affected animals in one breed to as many as 78 percent in another (Figure 111). It was also established that in any one breed some families might be resistant and others susceptible to experimental infection.

**Fig. III**

*Comparative Susceptibility to Scrapie of 24 Different Breeds of Sheep*

*Inoculum: Ten Percent Suspension of Scrapie Brain Tissue in Saline*

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number Inoculated</th>
<th>Affected With Scrapie</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herdwick</td>
<td>36</td>
<td>28</td>
<td>78</td>
</tr>
<tr>
<td>Dalesbred</td>
<td>43</td>
<td>31</td>
<td>72</td>
</tr>
<tr>
<td>Swaledale</td>
<td>46</td>
<td>25</td>
<td>54</td>
</tr>
<tr>
<td>S. S. Cheviot</td>
<td>45</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>Derby Gritstone</td>
<td>46</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>Exmoor Horn</td>
<td>41</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Border Leicester</td>
<td>42</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>Scottish Blackface</td>
<td>44</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>South Devon</td>
<td>35</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Romney Marsh</td>
<td>43</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Welsh Cheviot</td>
<td>40</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Ryeland</td>
<td>34</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Dorset Horn</td>
<td>45</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Suffolk</td>
<td>51</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Leicester</td>
<td>42</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Welsh Mountain</td>
<td>42</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Hampshire Down</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>N. S. Cheviot</td>
<td>45</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Southdown</td>
<td>38</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Wiltshire Horn</td>
<td>57</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Kerry Hill</td>
<td>41</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clun Forest</td>
<td>52</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dorset Down</td>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Incubation periods from time of inoculation ranged from 3½ to 23 months.
Period of test: July 1957 to July 1959.

This information makes it clear that one must be cautious in assessing the results of scrapie transmission experiments. Not only must the inoculum used be infective but the recipient animals must be susceptible; otherwise, transmission fails. Again this suggests that the genetic constitution of the recipient animals is an important factor in determining infection. The application of this knowledge to experiments in studying the methods of spread of the disease by contact or by any other method is obvious.
(5) Transmission of Scrapie, Experimentally, from Parents to Offspring

There is much evidence from field observations that either an infected father or an infected mother may sometimes produce affected offspring. It has been proven at Compton that there is a significant correlation between the susceptibility of mothers and their offspring to experimental infection. This correlation, however, although significant is by no means absolute. The correlation between the susceptibility of fathers and their offspring has not yet been proven experimentally but there is good field evidence that affected rams can sometimes produce affected offspring. When both parents are affected, present evidence suggests that the offspring are more liable to develop the disease. Even here disconcerting results can be obtained as illustrated by the following observation. A ram and ewe which were the progeny of parents that both died of scrapie were mated and produced three crops of lambs. The ewe and ram developed scrapie when they were two years eight months and two years 10 months old respectively. The second crop of lambs, a male and female twin, both developed scrapie when they were two and one-half years old. At this time, the first crop which consisted of one castrated male lamb was still apparently normal, however, when he reached the age of four years two months he developed scrapie. The third crop which was one female has now developed scrapie when almost four years old.

It is possible that these three crops of lambs acquired the disease hereditarily but since they were all reared in a scrapie contaminated environment, it is equally possible that they were susceptible animals and that they picked up infection at different ages.

---

**Figure IV**

*Occurrence of Scrapie in the Progeny of Inoculated Parents*

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Ewes</th>
<th>Rams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ten percent scrapie brain suspension in saline</td>
<td>140</td>
<td>4</td>
</tr>
<tr>
<td>Affected with scrapie</td>
<td>45</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Offspring Not Inoculated</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total born</td>
<td>59</td>
<td>64</td>
</tr>
<tr>
<td>Affected with scrapie</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

In another experiment, Figure IV a flock of 140 Cheviot ewes and four rams were inoculated with the scrapie agent 20 days after they had been mated. Two of the rams and 45 of the ewes developed scrapie. Lambs were born to these inoculated parents when scrapie was prevalent amongst the ewes and of 123 lambs born, nine developed scrapie when they were seven to 18 months of age. The mothers of seven of these lambs died of scrapie, and the mothers of the remaining two have remained healthy so far. Unfortunately, it was not known which of the four rams was the sire of the
affected lambs, and so another experiment is in progress in which the susceptibility of both parents to experimental infection will be known.

(6) Transmission of Scrapie to Goats

It has previously been reported from Compton that scrapie can be transmitted to goats and passed in series indefinitely through these animals. (Figure V shows an experiment in progress at Compton by I. H. Pattison.)

**Figure V**

*Passage of Scrapie Through Goats (Pattison I. H. in Progress)*

<table>
<thead>
<tr>
<th>Months After Inoculation</th>
<th>6.8.54</th>
<th>18.1.56</th>
<th>16.11.56</th>
<th>2.7.57</th>
<th>22.4.58</th>
<th>6.1.59</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>12 Goats</td>
<td>26 Goats</td>
<td>6 Goats</td>
<td>6 Goats</td>
<td>6 Goats</td>
<td>4 Goats</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td></td>
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<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>11</td>
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<td></td>
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<tr>
<td>12</td>
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<tr>
<td>13</td>
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<td>14</td>
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<td>15</td>
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<td>16</td>
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<td>17</td>
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<td>18</td>
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<td></td>
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<tr>
<td>19</td>
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<td></td>
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<tr>
<td>20</td>
<td></td>
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<td></td>
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<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Affected</td>
<td>12</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

It can also be passed from goats back to sheep again producing the classical symptoms of scrapie. The indications are that provided scrapie brain is used as inoculum and provided the inoculation is made intracerebrally, the "take" of scrapie in goats will be 100 percent of animals inoculated. This
finding will make much work on scrapie, which was hitherto difficult or impossible in sheep, a practicable proposition in goats. Already experimental results indicate that if an inoculum prepared from brain tissue is treated by physical or chemical processes, or if the inoculum is given by a route other than injection into the brain, the "take" of scrapie may be reduced, and the incubation period may be lengthened. It has also been found that the transmissible agent can be detected in the cerebro-spinal fluid 24 hours after experimental infection, and that it can be recovered as the disease progresses from the brain, pituitary, adrenal gland, spleen, pancreas, liver and sciatic nerve in that order.

Further work in progress aims at defining more perfectly the pathology, natural method of spread, immunology, diagnosis, methods of prevention and control or eradication of the disease.

The fact that the growing point of spread of scrapie infection in the United States appears to be confined to one breed of sheep suggests that the present eradication programme may be effectively blocking the excursion of infection into other breeds. The elimination of sources of infection in a country in which the disease has comparatively recently made its appearance must surely result in a reduction in the spread of infection, and if the programme is relentlessly pursued, and if necessary revised, as fresh information dictates, eradication may yet be achieved.

REFERENCES


Doctor Marsh: Thank you, Doctor Gordon. We should all realize that these men are simply summarising a great deal of research work they have done over the years. Next we will hear from Doctor Stamp, Director of Moredun Institute in Scotland. Doctor Stamp.
DR. J. T. STAMP, D.Sc., M.R.C.V.S., F.R.S.E.,

_Director — Moredun Institute, Scotland._

Doctor Marsh, Ladies and Gentlemen of the United States Livestock Sanitary Association: Scrapie disease in sheep has been present in Great Britain for very many years. At the present time it doesn't seem to give rise to serious losses among our own sheep flocks as a whole, but from time to time it has presented a serious problem to individual flock masters. I think one of the most important factors, in recent years, has been the interference with the export of pedigree sheep from Britain to both Canada and the United States. This is unfortunate for both Britain and the United States of America as for the former country it means a considerable loss of national prestige since Britain regards herself as being unrivalled in the breeding of good stock while the sheep breeders of the United States on occasion desire to import British blood ewes.

Unfortunately, the present resume of the work we have done does not give the answer to the scrapie disease problem, but it does set out the research work done at Moredun Research Institute in Scotland.

Some facts are indisputable. In the first place, scrapie can be made to occur in Cheviot and Scottish Mountain Blackface sheep when brain, spleen and lymph glands taken from sheep affected with scrapie are inoculated either intracerebrally, subcutaneously or intradermally into experimental sheep. The disease occurs five months or more after the inoculation. Further,

**Fig. A**

_Tissues Tested—(Other Than Scrapie Sheep C. N. S.)_  
 Route of Inoculation is Intracerebral, Unless Otherwise Stated

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of Cases</th>
<th>Inoc.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Tissue Pool</td>
<td>6</td>
<td>10</td>
<td>Lymph Glands, Spleen and Skin</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>Lymph Glands, Spleen and Skin S. C.</td>
</tr>
<tr>
<td>2nd Tissue Pool</td>
<td>1</td>
<td>10</td>
<td>Lymph Glands, Spleen and Skin</td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
<td>9</td>
<td>As Used in 1st Tissue Pool</td>
</tr>
<tr>
<td>Cerebro-Spinal Fluid</td>
<td>3</td>
<td>9</td>
<td>As Used in 1st Tissue Pool</td>
</tr>
<tr>
<td>Lymph Glands</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

it is known that Cheviot lambs develop the disease before they are nine months of age if they are inoculated at birth, this is an age at which natural scrapie never occurs. On the other hand similar sheep inoculated with brains taken from normal sheep failed to develop the disease except on one occasion.

The scrapie agent has also been successfully passed blindly at two-month intervals through seven groups of Cheviot sheep, so that it would appear that the agent is self-replicating. The results of dilution and filtration experiments
are also consistent with what one might expect with a biologically active particulate agent such as a virus.

One feature of the Moredun experiments with sheep is that the incidence of the experimental disease is always low averaging out to about twenty-seven percent of the inoculated animals. Only on one occasion has the incidence been 100 percent, and that is when the sheep that were inoculated were the offspring of a scrapie affected ram.

There is on the other hand some evidence that might suggest that the scrapie agent is not a virus, at least of the type which we at present recognize. It is evident that the disease can be reproduced experimentally in series when the agent has been boiled at 100 degrees centigrade for 8 hours, or when it has been heated at 96 degrees centigrade for 24 hours.

**Fig. B**

*Physical Treatments of Scrapie Sheep C. N. S.*

*Route of Inoculation is Intracerebral, Unless Otherwise Stated*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cases</th>
<th>Inoc.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoclaving 20 lbs. 30 mins.</td>
<td>0</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Boiling for 30 mins.</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>Lambs</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>50</td>
<td>Boiled Scr. C. N. S. +++ Unboiled Normal C. N. S. (Lambs)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>50</td>
<td>Boiled Scr. C. N. S. +++ Boiled Normal C. N. S. (Lambs)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td><strong>Dilutions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>19</td>
<td>10^-1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20</td>
<td>10^-2</td>
</tr>
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<td>1</td>
<td>20</td>
<td>10^-3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>29</td>
<td>Boiled Passage (2nd)</td>
</tr>
<tr>
<td>Boiling for 8 hrs.</td>
<td>1</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Equally clear is the fact that the transmissible factor is present in about equal quantity in both boiled and unboiled suspensions. In other words the boiling does not seem to decrease to any great extent the quantity of material present in the suspension. In addition to the agent being resistant to heat it is known that it can withstand the action of three percent formalin for 13 days at 37 degrees centigrade, or 0.8 percent formalin for four months. The agent is also resistant to the action of acetyleneimine
which is known to destroy nucleoprotein. On the other hand it will not
withstand autoclaving at 20 pounds per square inch pressure for 30
minutes.

We have attempted to purify and concentrate this agent by butyl alcohol
fractionation techniques similar to those that have been applied to the smaller
viruses such as poliomyelitis but without success since the agent was found
to be present in both gel and aqueous phases.

It can be seen therefore that the evidence as to the etiology of scrapie is
difficult to interpret. On the one hand the transmission experiments suggest
that scrapie is an infectious disease caused by a virus whereas the resistance
of the agent to heat and chemical agents, et cetera, make it unlikely that the
factor is a typical virus particle.

### FIG. C

**Chemical Treatments of Scrapie Sheep C. N. S.**

*Route of Inoculation is Intracerebral, Unless Otherwise Stated*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cases</th>
<th>No. Inoc.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formalin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 percent at 37° C. for 13 days</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1.5 percent at 37° C. for 14 days</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>0.8 percent at 40° C. for 2 months</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>0.8 percent at 4° C. for 4 months</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td><strong>Formalin 1 percent and Ether 1 percent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37° C. for 14 days</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

| **Chloroform**                   |              |           |
| 5 percent at 37° C. for 13 days  | 2            | 10        |
| 1 percent at 37° C. for 14 days  | 3            | 12        |

| **Phenol**                       |              |           |
| 2 percent at 37° C. for 13 days  | 3            | 11        |
| 1 percent at 37° C. for 13 days  | 3            | 11        |

### FIG. D

**Physical Treatments of Scrapie Sheep C. N. S.**

*Route of Inoculation is Intracerebral, Unless Otherwise Stated*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cases</th>
<th>No. Inoc.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Freezing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—70° C. for 2 months</td>
<td>4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>—40° C. for 2 months</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>—30° C. for 20 months</td>
<td>3</td>
<td>11</td>
<td>Boiled before inoc.</td>
</tr>
<tr>
<td>—30° C. for 17 months</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>—20° C. for 2 months</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

| **Drying**                     |              |           |                     |
| 4° C. in Desiccator over       | 3            | 4         |                     |
| CaCl₂ 2½ years                 |              |           |                     |
Although numerous attempts have been made to reproduce scrapie in the mouse, the rat, the guinea pig, and in tissue culture, this has not been achieved. In addition no evidence of an antigen or an antibody has been found even though all known serological techniques including fluorescent antibody techniques have been tried.

We have done a lot of work with the electron microscope but so far have failed to reveal any virus particle in scrapie material.

As a result of these conflicting findings it was decided to investigate the genetic aspects of scrapie and for this purpose a breeding experiment was started at our Institute in 1955 using the male and female offspring of scrapie affected sheep and the male and female offspring of clean sheep. These were bred in four groups in order to determine if the disease is familial and if so to provide information on the mode of transmission since a knowledge of the epidemiology of the disease is essential for rational field control. The four groups consist of dirty sheep crossed with clean sheep, dirty sheep with dirty sheep, clean sheep with clean sheep and clean sheep with dirty sheep, a two by two genetic experiment. From the results of this experiment up to the present it would seem very definite that if the parent has scrapie then the chance of its offspring having scrapie is relatively high. We have a considerable amount of evidence not only from our own experiments but also from the field that nearly all the offspring of a scrapied ewe will ultimately develop scrapie. It may take a long time for the offspring to go down with the disease but nearly all do so eventually. It would appear from our experiments that the incidence of scrapie is higher in the offspring of scrapied ewes than of scrapied rams. This we feel may be due to an unbalanced representation which we had in our experiments in the early years, but this will be rectified next year since we have a considerable number of offspring of scrapie affected sires coming along.

We think that it will be possible to state quite definitely at the end of the next 12 months what the position is regarding sires and their offspring.

In addition offspring have been bred from clean ewes inseminated with mixed semen, semen from a scrapie ram and semen from a clean ram. The lambs born to each sire can be distinguished by color marks. Again we feel that within the next 12 months this will give us an indication whether scrapie can pass from father to offspring and if so whether it goes through the genetic material or through the seminal fluids.

It could of course, be argued that the familial associations of scrapie which we have shown to exist are apparent rather than real since our scrapie offspring have been raised in a "scrapie infected" environment and our clean sheep have not. As a control to this we have taken a number of clean sheep away from the experiment and put these clean sheep in contact with as many scrapie animals as possible, and up to the present time none of these animals has taken scrapie. As a further control we have kept one hundred young Cheviot sheep for five years, in contact with clinical cases of scrapie, and no cases of scrapie have occurred in these contact sheep. These two results are in contradiction to the results which Doctor Greig published some time ago, but I feel that our experiments, although they have been running for four and
one-half years are far from complete and I will be willing to change my mind if factual evidence comes forth in the next few years that contact infection does occur.

Considerable work on the pathology of scrapie has shown that the characteristic and most consistent lesions are in the neurones of the brain stem and cerebellum although astrocytic proliferation can also be seen. All the neuronal lesions consist of various stages of degeneration and/or vacuolation of the cytoplasm. The medulla and pons are the most convenient parts of the C.N.S. for diagnostic examination. The lesions in the medulla can be found in any of the cell groups or nuclei but the following nuclei are more commonly affected than others.

1. Arcuate
2. Raphe
3. Reticular formation
4. Dorsal motor vagus
5. Cuneate
6. Red nucleus

The numerical difference in the incidence of vacuolated neurones between scrapie animals and apparently healthy sheep is very obvious when serial sections of the medulla are examined.

No evidence could be obtained that muscle lesions are a characteristic finding in scrapie disease.

Doctor Marsh: Thank you very much, Doctor Stamp. I think you see from the remarks these men made that they are reporting on a very large amount of work in a very short time and we certainly appreciate them giving all of these points in such a concise manner.
DR. W. J. HADLOW: Thank you, Doctor Marsh. Gentlemen: I have just a few brief remarks to make about some of the observations I have made while working at Doctor Gordon's laboratory. At Compton work on the pathology of this disease has been extended in an attempt to find a morphologic basis for what is seen clinically and to remove scrapie from what in my view is the clinical wilderness in which it has remained for so long.

Most of my observations have been made on experimentally affected goats. It has become more apparent with passage in this species that the syndrome produced in the goat is essentially like scrapie in the sheep. The incubation period has been variable, but at present it is about eight to 12 months. However, it may be considerably longer, sometimes up to 30 months. All of the clinical signs usually associated with the disease in sheep may be seen in affected goats. Aside from wanting to characterize the experimental disease clinically, my main interest in studying the goat was to associate the various clinical signs with morphologic changes somewhere in the body. Thus far, significant changes have not been found outside of the central nervous system. I should like to corroborate what Doctor Stamp has mentioned about the absence of significant lesions in the skeletal musculature; scrapie is not a myopathy but is a disease of the central nervous system.

Fig. 1—Lateral geniculate Hadlow’s body. Azure eosinate stain.
(a) Normal goat.
(b) Goat affected with scrapie. Characteristic degenerative changes consist of shrunken, deeply stained nerve cells and an increase in number of astrocytes. Compare with 1 (a).
As observed in goats, the essential lesion consists of widespread degeneration of nerve cells. This degeneration characterized largely by shrinkage and increased basophilia of the neurons. Vacuolation of the cytoplasm of nerve cells usually occurs as another manifestation of the degenerative process. At least, I have interpreted it as an integral feature of the degeneration. Although vacuolated neurons may be seen at all levels of the neuraxis, they seem to be most conspicuous in the brain stem. Certainly, their presence usually is much more readily appreciated in the medulla oblongata and pons than elsewhere. This is not to say that the most severe lesions are found in the brain stem. In addition to degeneration of nerve cells, pronounced astrocytosis is regularly seen. By astrocytosis I mean an increase in the size and in the number of the astrocytes. In some areas of the brain, this glial response may be much more conspicuous than the evident neuronal damage. Thus, from a morphologic standpoint, these two features—widespread degeneration of nerve cells, and concomitant or secondary astrocytosis constitute the main lesion. These changes are essentially non-specific, a point to be emphasized.

The distribution or pattern of changes in the brain and spinal cord is of prime interest in any disease of the nervous system, including scrapie. Once this is determined, one can endeavor to make some sense of the clinical picture. With this in mind I embarked on a detailed study of the lesion in the central nervous system of the goat. I don't have slides today to illustrate this.
feature of the disease, but perhaps if I enumerate the areas that are most severely affected you will gain some idea of the distribution of the lesion.

Of all structures that may be involved, those in the diencephalon have been the most severely affected. Specifically, the various nuclei of the thalamus exhibit the most consistent and most severe changes. For example, thalamic nuclei forming part of the visual system usually are affected. Changes here would seem to be the morphologic basis for the visual impairment which is evident in many goats, particularly in advanced stages of the disease. Elsewhere in the visual system, lesions have not been found. In addition to changes in the thalamus, other structures in the general area that are known to influence motor activity also may be affected. One that deserves mention is the subthalamic nucleus or corpus Luysi. It is a small structure but presumably is of great functional significance in an animal such as the goat or the sheep.

Moreover, certain structures in the midbrain may be affected. Also, in the cerebellum both the cortex and the cerebellar nuclei often are affected by the degenerative process. Doubtless, damage of these structures would provide part of the basis for the tremor that occurs in many of the animals. As has been mentioned today and on many occasions previously, the pons and medulla oblongata also are involved. In the material I have examined, particularly from the goat, over-all changes here usually have not been striking, and certainly have never been as severe as those observed in the more rostral structures. However, as mentioned earlier, vacuolation of nerve cells probably is more readily appreciated in the pons and medulla oblongata than in other affected parts.

To reiterate, scrapie, as I have observed it in the goat and in far fewer sheep naturally or experimentally affected, is a disease of the central nervous system. Furthermore, it is a progressive degenerative neurologic disease characterized by essentially nonspecific changes which have an interesting pattern of distribution. In view of the limited information about the neurophysiology of the goat or the sheep, a restricted correlation of the individual clinical signs with this pattern of the anatomic changes is not possible at this time. Nevertheless, when viewed broadly and in general terms, the pattern of the lesion observed in the goat will seem to go a long way in helping to explain much of what is observed clinically in this perplexing disease called scrapie. Thank you.

DR. H. MARSH. Thank you, Doctor Hadlow, for explaining the nerve tissue lesions you have observed in scrapie in sheep and goats.

Next we will hear from Doctor Hourrigan who is in charge of the control and eradication of scrapie in the United States, Doctor Hourrigan.
Thank you, Doctor Marsh. Members of the United States Livestock Sanitary Association, Ladies and Gentlemen:

After listening to the three gentlemen preceding me I am sure you will agree that there isn't much left to say about scrapie. They have covered the field. I will mention briefly the eradication program and experiences here in this country.

The first report of scrapie was from Michigan in 1947. The second report was here in California in 1952 when 21 sheep in two related flocks were found to have the disease. At that time your Association asked the Secretary of Agriculture, Mr. Brannon, to take immediate action to eradicate the disease. To provide the means for doing so the Secretary declared an emergency on October 31, 1952, and thus the Scrapie Eradication Program came into being.

Like all animal disease eradication programs in this country it was, and is, a state-federal cooperative program with both having responsibilities.

It was recognized that scrapie was a particularly difficult disease to combat and that practical as well as effective program procedures were needed.

The procedures instituted were actually very similar to those now being followed in the program. They included quarantine and slaughter of infected flocks and slaughter of exposed sheep moved from them and their immediate progeny. Vehicles and premises were cleaned and disinfected and owners received state and federal indemnity for sheep slaughtered. Owners slaughtered many commercial fat lambs without indemnity. These procedures seem to have worked out quite well as more than seven years have passed and there has been no extension from these outbreaks. California has since suffered six additional outbreaks not associated with those in 1952.

Most of you are familiar with the efforts of industry representatives and regulatory officials to develop the best program possible to prevent scrapie from becoming widespread in the United States.

In this regard it may be of interest to review some of the highlights that have taken place since 1952.

There were, of course, several meetings during 1952 and 1953. In April 1954 research and regulatory workers met in Washington, D. C. to discuss scrapie.

The following month a Washington meeting was held and members of the sheep industry and state and federal livestock sanitary officials met to discuss this disease.

As a result of the meetings just mentioned federal indemnity was increased in July 1954 to one-half the difference between the appraised value and
net salvage value not to exceed $25.00 per head for grade sheep and $75.00 for purebred sheep.

In May 1956 research workers, livestock sanitary officials and Canadian officials met in Washington, D. C. to review the program and in August of that year meetings were held at St. Louis, Missouri, with the National Suffolk Sheep Association and other members of the industry.

In November, 1956 a meeting with a group of consultants was held in Washington to consider the eradication program and research needed.

In June, 1957, several research and regulatory officials met with the National Suffolk Sheep Association at Columbia, Missouri.

In July, 1958 a similar meeting of the National Suffolk Sheep Association and regulatory officials was held in Washington, D. C.

In November, 1958, a Scrapie Study Group was selected to study the scrapie problem. Your Association had recommended that a study be made. I am sure you are all familiar with the report of this study group as it was duplicated and distributed.

On numerous occasions then, members of industry, research workers, staff members of veterinary colleges, regulatory officials, and others met to discuss the scrapie problem and the program. As many as could traveled to Britain and France to visit sheepmen and research institutions and consult personally with authorities on the disease.

We were very pleased when we learned Dr. J. T. Stamp, Dr. W. S. Gordon, and Dr. W. J. Hadlow could come and share with us directly their considerable experience and knowledge. As many of you know, they have given lectures at Washington; at Chicago where an industry meeting was sponsored by the National Wool Growers Association; at Purdue University; Ohio State University; Denver, Colorado; Sacramento, California; the University of California at Davis; and of course here as guests of your Association.

Several Canadian sheepmen attended the Chicago meeting and were interested in having our visitors appear in Canada and Dr. K. F. Wells, Veterinary Director General in Canada made the necessary arrangements for a meeting at Lethbridge, Alberta.

Following the first California outbreak the disease struck in Ohio and Illinois. In Ohio the eradication program included slaughter of infected flocks but did not require slaughter of exposed sheep moved from them or their immediate progeny. Certain such animals were later found to be infected in Ohio and in Tennessee. In Illinois the infected flocks and exposed sheep sold from them were slaughtered.

A Canadian flock appeared to have been involved in the California, Ohio, and Illinois outbreaks.

These experiences further emphasized by a series of outbreaks mostly in Indiana, furnish information that scrapie spread from one flock to 13 others in four states; that another flock had disseminated the disease into six flocks in two states; and two others into ten flocks in four states.

It was with this background in mind, and following meetings and consultations, that the national cooperative eradication program was broadened
in April 1957 to include slaughter of source flocks and exposed sheep moved from infected and source flocks and their immediate progeny. Source flocks were defined as those found, with ample evidence and after careful consultation, to be disseminating the disease but in which no animals showing clinical symptoms of scrapie could be demonstrated. Several states had previously found it necessary to adopt these procedures and had done so.

Regulatory officials in this country have worked closely with those in Canada as obviously the scrapie problems are similar and are interfused.

In August, 1959, the Canadian Scrapie Eradication Program was similarly broadened so that Canada and the United States now have equivalent eradication programs.

The broadened Canadian program should materially reduce and we hope eliminate the opportunity for introduction of scrapie through imported sheep. We are cooperating very closely in all phases of the scrapie problem.

Scrapie has been reported in England, Scotland, Wales, France, Germany, Austria, Hungary, Poland (Prussia), Spain, Norway, Iceland (where it is called Rida), Australia, New Zealand, Canada, and the United States.

In this country 85 infected flocks have been reported in 70 counties in the following 22 states: Alabama, California, Connecticut, Georgia, Illinois, Indiana, Iowa, Kentucky, Michigan, Mississippi, Missouri, New York, North Carolina, Ohio, Oregon, Tennessee, Texas, Utah, Virginia, West Virginia, Wisconsin, and Wyoming.

Following the 1947 Michigan outbreak no additional infected flocks were reported until fiscal year 1953 when ten were disclosed. There were three in 1954; 11 in 1955; 23 in 1956; 12 in 1957; seven in 1958; 11 in 1959; and seven to date in fiscal year 1960.

Flocks from which exposed sheep and/or their immediate progeny are removed are inspected at least every six months for 42 months to determine if scrapie was introduced.

Such sheep have been removed from approximately 1,600 flocks. There is no evidence that this procedure has resulted in additional outbreaks. There were outbreaks where this was not done.

Presently some 1,300 flocks are receiving the required six months inspections. In November, 1956 more than 2,000 flocks were in this category.

Almost 50 percent of the 85 outbreaks were found in this manner—many issuing from the period when source flocks and exposed sheep from infected or source flocks and their immediate progeny were inspected rather than being slaughtered.

Practicing veterinarians have reported almost one third of the outbreaks.

Two of the 85 outbreaks were disclosed when infected sheep were sent to market and there alert federal inspectors at Public Stockyards observed symptoms and suspected scrapie.

Owners have been cooperative and in numerous instances reported evidence of disease in their sheep. Sheepmen who were familiar with scrapie symptoms as a result of public meetings and veterinary inspections of their flocks notified officials of suspicious symptoms or took sheep to veterinary institutions.
Doctor Gordon and Doctor Stamp tell us that scrapie is not limited to one or two breeds of sheep and that the history of scrapie indicates it spreads from one purebred breed to another. If one accepts registration certificates at face value it is difficult to explain such spread on a parent to progeny basis.

Of the 154 confirmed cases in this country 145 were Suffolks and 9 were Cheviots. Cases in Canada have been more evenly divided between these two breeds. One infected Canadian flock included both purebred Suffolk sheep and purebred Hampshire sheep. Scrapie was diagnosed in both breeds. The same was true of another flock that included both purebred Suffolk and purebred Southdown sheep.

The 85 outbreaks in the United States illustrate the potentiality of an insidious disease like scrapie in a country such as ours where the lively trade among growers results in wide distribution of sheep and diseases affecting them.

We look forward to additional research, particularly that designed to explain natural transmission. Precise information in this regard will permit more exact identification of potentially dangerous animals and, it is hoped, provide for more effective eradication procedures requiring slaughter of fewer sheep. This knowledge is especially important in absence of a diagnostic test.

We cannot look into a crystal ball and foretell what will happen during the next three or four years. But experience is convincing. The elimination of infected and exposed animals has effectively controlled the disease, and prevented its becoming widely established not only in Suffolk and Cheviot sheep but in other breeds as well.

The chances for ultimate success appear good if a sound eradication program is followed supported by ample research.

SCRAPIE PANEL QUESTIONS

DOCTOR MARSH: Thank you, Doctor Hourrigan. We now have a little time for questions that you may wish to propose to these gentlemen. Has anybody any question he would like to ask?

DR. A. K. KUTTLER: I would just like to have someone comment on why the incidence of the disease has been so high in this country and so relatively low in Europe?

DOCTOR GORDON: I think in the first place one should make it clear at one time scrapie did not occur in the Suffolk breed in England. This is just an example of the way in which this disease moves. It has now created a threat to the Suffolk breed. It is supposed to have gotten into the Suffolk breed by the exportation of half breed ewes, that is a cross breed of Border-Leicester and Cheviot sheep from Scotland. Half breed ewes being transferred to England to the eastern counties which are the home of the Suffolk breed. Well now, if this disease does not spread by contact, then I leave it to your own imagination as to how it got into the Suffolk breed. I am afraid I am at a loss as to how this would take place. But it is quite clear that right after the
war the disease did creep into the Suffolk breed and unfortunately it got in amongst some of the ram breeders flocks and there was one leading flock, I believe, and this information once again was presented to me by a person who is associated with the Suffolk breed who was trying to convince me that if the disease got into the Suffolk breed it got in by contact, but anyway the owner died and his flock was sold. At the dispersal sale many of the Suffolk breeders purchased stock. That is the way it got into the Suffolk breed and the manner in which it was dispersed by that breed.

Relative to the question of spread by contact. This is a very difficult question to answer. As I said when I was talking, I at one time inoculated 30 Cheviot sheep intercerebrally with the scrapie agent and about 60 percent of the inoculated sheep took scrapie. Living alongside of these sheep was another group of 30 which were not inoculated. Four years 42 months from the start of that experiment one of the noninoculated sheep in contact with the inoculated group developed scrapie. In another experiment we had a group of sheep inoculated with normal brain material as well as sheep inoculated with scrapie infected material and after they had been in contact for a period of over two years several cases of scrapie developed in the sheep inoculated with normal brain material. Also a group of sheep inoculated with saline into the brain and had been in contact with scrapie infected animals, we got several cases of scrapie. Now it may be that those sheep that took scrapie in the contact group were destined to take the disease naturally. But of these two possibilities the one that I favor in connection with these experiments was the spread by contact. And lastly the most recent thing that has happened is that we have fed a goat with brain material from a scrapie infected animal and this goat 15 months later has taken the disease. If we can confirm this by a number of other experiments, for purposes of confirmation, it would make the possibility of spread by say ingestion or contact more acceptable. But I still contend that the most important method of the spread of this disease is through families. The progeny of scrapie parents are very likely to take scrapie but not all progeny of affected parents do take the disease. There is significant correlation between infection in the mother and susceptibility of the mother and susceptibility of the offspring.

Mr. Spencer, Wilton, California: May I ask the Doctor, You are satisfied that scrapie is a blood disease?

Dr. W. S. Gordon: I am not satisfied that it is a blood disease. We are pretty well satisfied that the transmissible agent is present in the central nervous system of sheep. Its presence can also be demonstrated in lymph nodes, adrenal, pancreas, spleen and some other tissues. It is fairly widespread in the infected animal. But so far we have not been able to recover or demonstrate the agent in the blood of affected animals.

Mr. Spencer: Timing is one other question that you have to look to. Where yearlings are going to break with scrapie at two or three months or at two and one half years. That is the timing situation you are faced with which constitutes a real problem. Well that is the reason I felt we could connect it up with blood lines. If a blood situation might come from a fly. I would
not imagine if there were any flies in the country spread would be any faster than it is now.

**Doctor Gordon** said no he did not think so.

**Dr. Vawter**, Corvallis, Oregon: Would it be any advantage that when you see an animal that is showing unmistakable clinical signs of scrapie that you place that animal in quarantine and segregation away from any possible contact with other animals and hold that animal somewhat longer for a period of two to three weeks until full symptoms develop and in that manner allay any possibility of confusion in regard to whether that could be positive for scrapie on histologic examination. I am simply advancing that in view of our own experience in the last six months.

**Dr. W. J. Hadlow:** Well I would agree with you that the lesions are much more appreciated in a well advanced stage of the disease. Certainly in studying the disease in the goat the lesion is rather subtle and unless you have an adequate base line of normal sections it is easy to overlook it, but this is less easily done in advance stages of the disease. I am talking now about the over-all degeneration of nerve cells and the appreciation of changes in structures other than the medulla and pons. But I think Doctor Stamp has some observations on the relative ease of making a diagnosis in various stages of the course of the disease.

**Dr. T. J. Stamp:** I think that if you let the disease progress you get a more obvious pathological picture. I mean it would be a strange disease if this didn't happen. On the other hand we are quite convinced at Moredun that with our experience with the diagnosis of scrapie, if an animal is in fact showing signs of scrapie we can confirm our diagnosis there and then by pathological examination, by the counting of vacuoles, by the checking of the number of vacuoles against what we know as our base line as Doctor Hadlow describes it. Up to date we have no difficulty in confirming our diagnosis even quite early in the disease, and we have now examined many hundreds of scrapie sheep.

A **man** from California: I would like to hear Doctor Gordon describe the clinical symptoms of scrapie.

**Dr. W. S. Gordon:** There are two predominating symptoms in scrapie. The one is the characteristic "itch" symptom which may be more intense in some stages in the course of the disease. But as the disease progresses the appearance of disorder of function of the central nervous system becomes obvious and you get some characteristic symptoms like when the affected animal is forced to move, it gallops behind and trots in front. Also, it begins to cross its legs and show some locomotor disturbance.

In some breeds of sheep we have found that the most outstanding is the symptom of locomotor disorder and the itching is not so manifest but in natural cases of scrapie I pin a great deal of faith on getting the animal some object that it can rub itself upon and then observe it when it doesn't realize you are there and then also when you handle it and rub it it shows pleasure at this attention. I think these are the two main symptoms, a combination of the "itch" phenomenon and the locomotor symptom.
If you are just patient and take your time the sheep will convince you that this is the disease it has.

MR. FARLEY of California: What step could the livestock industry take with regard to exportation of sheep?

DR. W. S. GORDON: Well, I have nothing to do with policy. But in England there are 36 different breeds of sheep and each one of these registered societies has a president and a council. From that council they supply a member of the National Sheepbreeders Association. Now the National Sheepbreeders Association of Britain are most anxious to get the export market opened again. They have formed a committee which has been pressing the Ministry of Agriculture to try to make some arrangement for the screening of flocks that might wish to export sheep. No arrangement has been come to so far and whether one will be found I just can't say. But the kind of thing they have been thinking about is that once a pedigree flock had been under surveillance for say three or four years without any cases of scrapie occurring in the flock and the only intertrade that these flocks could engage in would be between themselves. They might form a scheme where reliance could be placed upon animals that are sold from such flocks. This, I think, is an extremely difficult thing to try to arrange. It is also very unwise for me to discuss this at great length since I have nothing to do with the formulation of such a program and so I am afraid I cannot give you any definite answer to your question but only say that I do hope some method is found whereby some of the breeds of sheep of Britain will become acceptable to other countries again.

VOICE: Who bears the loss from scrapie in Britain?

DOCTOR GORDON: So far scrapie has not been nationalized in Britain and so the loss has to be borne by the farmer himself. Sometimes this loss can be very heavy in a pure bred flock both from deaths and eventually from loss of sales due to flock having a reputation for scrapie.
The Committee on Infectious Diseases of Sheep and Goats is a new committee set up since the 1958 meeting of this Association, because the officers of the Association decided that more time and attention should be devoted to diseases of sheep which create regulatory problems.

The Committee recommends that the word “infectious” in the name of the Committee be changed to “transmissible,” as it seems desirable to go beyond the strictly infectious diseases in some cases, even though there may be overlapping with the field of the general committee on parasites.

In this initial discussion of the infectious diseases of sheep and goats, we have selected several diseases which we think should receive consideration at this time by the regulatory officials. This is not to say that these selected conditions are the only ones which need consideration from the control standpoint, but a discussion of them seems particularly appropriate at this time. The Committee will attempt to briefly call attention to the available information pertinent to possible control procedures for this group of diseases, and will make some recommendations for action.

FOOT-ROT

The first condition which we will discuss is a sheep disease which has been with us a long time. In the opinion of this Committee it is time to wake up and do something about foot-rot. In the past few years letters from several states have come to the chairman of this Committee asking what can be done about foot-rot. A letter recently received from a man who has sheep contacts in several eastern states indicates that he thinks foot-rot is the controlling factor in maintaining or expanding the farm flock business.

Correspondence indicates that many veterinarians do not fully understand the foot-rot problem or its treatment. This situation should be corrected, as the necessary information is available. Foot-rot is a non-suppurative disease of the corium of the hoof, characterized by progressive necrosis of that tissue, resulting in separation of the horn of the wall and sole from the tissues beneath. It must be distinguished from suppurative foot infections which usually extend to tissues above the hoof. In Australia the causative agent has been shown to be *Fusiformis nodosus*, and in that country laboratory diagnosis is made on finding the organism in smears. The only known source of the infection is the infected sheep. The infective agent does not remain...
viable on the premises for more than a few days after infected sheep are removed.

Systemic treatment with sulfonamides or antibiotics has not been effective. There is no immediate prospect of control by immunization. But the disease can and should be eradicated from an infected flock. This can be done by the following procedure: Every foot in the flock is examined and all affected sheep are separated from those showing no infection. The clean sheep are walked through a foot-bath of 30 percent copper sulfate or five percent formalin and moved to a dry pasture where the infected sheep have not been for 30 days. The affected feet are thoroughly trimmed to expose all diseased tissues and treated by standing in 30 percent copper sulfate or 10 percent formalin. In Australia a 10 percent alcoholic solution of chloromycetin has been recommended for treatment of affected feet. Following treatment, all affected feet should be re-examined after about one week and retreated if necessary. After a convenient interval the entire flock should be re-examined.

In Montana every known infected flock is quarantined until the state veterinarian declares it free from infection. In two of the Australian states no movement is allowed from infected flocks until after they have been treated and declared free by the inspectors, and in Victoria all sheep must be inspected for foot-rot twice a year.

This Committee strongly recommends that all State Livestock Sanitary authorities make proper provision for diagnosis and quarantine flocks known to be infected with foot-rot and clean them up. A resolution on foot-rot control is being submitted to the Committee on Resolutions.

SCRAPIE

A regulatory problem of immediate importance in the area of diseases of sheep is the control and eradication of scrapie. In looking through the proceedings of the meetings of this Association, we find very little reference to scrapie. In 1952, the original California outbreak was reported, and a resolution was passed urging the Secretary of Agriculture to participate cooperatively with the states in the eradication of scrapie. In 1956, a resolution was passed requesting the Secretary of Agriculture to undertake research on scrapie, and to strengthen the regulatory requirements to control and eradicate scrapie.

The scrapie eradication program which was set up in 1954 was strengthened in some features in April, 1957. The program adopted at that time is still in force.

Under this program the finding of one infected sheep in a purebred flock has, in some instances, necessitated the slaughter of several hundred or thousand sheep in a considerable number of flocks. This situation naturally alarmed the breeders, more especially because nearly all the infected animals were of one breed. One breed association questioned whether eradication was being accomplished by what they felt was an unjustifiably drastic program. They requested a modification which would reduce the number of sheep slaughtered by requiring only the visibly affected sheep and their immediate progeny to be destroyed, except in exceptional cases.
In November, 1958, the Animal Disease Eradication Division of the Agricultural Research Service set up a series of hearings at five points in the country before a scrapie study group. At these hearings Doctor Hourrigan presented a resume of the available information on scrapie, and sheep breeders, regulatory veterinarians, and research men presented their experiences and ideas.

Although some segments of the industry were opposed to the eradication program as it was conducted, there was general agreement that some eradication procedure should be continued. The western representatives of the industry favored the continuation of the present program, while eastern representatives of the industry asked for modification of the procedures.

The study group, of which the chairman of this Committee was a member, gave very careful consideration to all the phases of the problem and to all the information presented by those attending the hearings. Some members of the study group felt at the start of the assignment that there should be a modification of the program, but when all the information, both from this country and Europe, was analyzed, the group decided they could not recommend a relaxation or modification of the procedure at that time. This decision was based partly on the fact that the program was apparently making progress toward eradication as the number of known infected flocks dropped from 23 during fiscal year 1956 to 12 in 1957 and seven in 1958. During fiscal year 1959 there was a slight increase in known infected flocks up to 11.

The study group recommended the amendment of import regulations to give the industry better protection from the apparent principal source of the infection, which has been the importation of sheep from Canada, where the control procedures have been somewhat less complete than those in force in the United States. On August 31, 1959, Canada adopted a scrapie eradication program which is the equivalent of the program in force in the United States.

The scrapie study group also found that adequate compensation for animals slaughtered on account of scrapie was an important factor in obtaining effective cooperation of the industry in the eradication program.

The development of a control and eradication program has been very difficult because of the very peculiar nature of scrapie. There is little evidence to support the genetic theory of the etiology. Research in Britain and France has shown that there definitely is an agent in the tissues of affected sheep which can produce the disease in sheep and goats when experimentally inoculated. In the experimental disease less than half of the sheep inoculated have developed the disease, while in goats the inoculations have been 100 percent effective, although goats have never been known to be affected naturally. The natural mode of transmission is unknown. There is little experimental evidence of transmission by contact. The infecting agent is unique in its resistance to boiling for several hours and to ordinary disinfecting chemicals. The long incubation period complicates control and makes research difficult and slow.

One of the recommendations of the study group was that research should be undertaken in the United States and in cooperation with European
countries, to obtain as rapidly as possible the information needed to make control and eradication procedures most effective.

Your Committee is of the opinion that the advisability of continuing the scrapie eradication program of the Agricultural Research Service as at present in operation should be re-evaluated now after another year of experience. Although the scrapie study group which examined the situation a year ago concluded that it could not recommend modification of the procedure at that time, there continues to be dissatisfaction with the procedure in some segments of the sheep industry and lack of indemnities by certain of the state governments involved interferes with the success of the program. While research has not yet resulted in developing much new information which can be used in control work, another year of control experience with opportunity for further analysis has passed.

In view of the somewhat controversial nature of the situation, which involves federal and state cooperative effort, your Committee is of the opinion that the United States Livestock Sanitary Association should examine the problem and take some action.

Your Committee therefore recommends that a committee of the United States Livestock Sanitary Association be appointed which shall include representatives of the sheep industry, the state veterinary services, and the Agricultural Research Service. It is recommended that this Committee review the history of the eradication program through the year 1959 and be authorized by the Association to make definite proposals to the Agricultural Research Service, the state livestock sanitary officials and the Canadian Health of Animals Division as to possible modifications of the program and the development of a fully cooperative effort. This Committee should have available the information gathered by the scrapie study group in 1958 and any additional information developed since that time.

Your Committee further recommends that the United States Livestock Sanitary Association urge the Secretary of the United States Department of Agriculture to increase the program of contributing personnel and funds to the existing scrapie research projects of England and Scotland, with the objective of accelerating research results. A resolution to this effect is being submitted to the Committee on Resolutions.

BLUETONGUE

Since the recognition of bluetongue in California and Texas in 1952, the disease has been reported from a total of 14 states. There is no reason to believe it will not appear in other states that have sheep. This is quite understandable when one considers that transmission of bluetongue is accomplished by blood-sucking insect vectors. The vector most frequently incriminated, Culicoides variipennis, is thought to be widely distributed.

The current procedures for diagnosis of bluetongue in states or areas where it is not officially recognized seem to be adequate. They are based on procuring blood samples from suspected sheep during the febrile stage of the disease and sending them to an institution or laboratory equipped to inoculate susceptible sheep and demonstrate the typical disease pattern. In
many instances, isolation of the virus on developing chicken embryos is also accomplished to further pinpoint the diagnosis.

Past and present experience indicates that this disease can be adequately controlled by vaccination. While there are some reports indicating an occasional severe vaccine reaction, there are no authenticated reports of properly vaccinated sheep breaking with the disease. Continued outbreaks of this disease are based upon man’s inherent ability to ignore sound disease prevention practices.

Although several states have justifiably placed restrictions on the importation of sheep from states where bluetongue is known to exist, the basic knowledge concerning the distribution of possible vectors, the state of carrier sheep, and reservoirs other than sheep is so slight that no sound, nationwide, regulatory recommendations could be made at this time. It is believed that this Association should go on record as strongly recommending increased research in the areas mentioned: namely, the distribution of suspected vectors and the identification of other vectors, investigations of the carrier state, investigations into all possible reservoirs of infections, other hosts, and investigations into the possibility of decreasing the vaccine reaction without seriously handicapping the immune response.

OVINE VIRUS ABORTION

Ovine virus abortion was first reported from the United States in 1958. A two-year study of the geographic distribution in this country of the disease was started in 1958 through a cooperative project of the Agricultural Research Service, Animal Disease Eradication Division, and the Veterinary Research Laboratory of the Montana Agricultural Experiment Station. The results of the first year’s study positively identified the virus as being present in the sheep population of eight states with two other states being classified as suspicious.

A differential diagnosis between virus and bacterial abortions cannot be made from symptoms or gross pathology. One must resort to stained tissue smears or serology. Microscopic examination of stained cotyledon smears showing elementary bodies, with a positive complement fixation or agglutination test constitutes a positive diagnosis; whereas, negative serological reactions with these antigens are obtained in the case of bacterial abortion. Bacteriological cultures should be prepared to exclude vibriosis or other bacterial etiologic agents.

There is no evidence that the ram is involved in the transmission of the infection. The transmission occurs at the time of parturition or abortion. Therefore, every possible sanitary precaution should be employed to minimize exposure to infected fetuses, placentas, and discharges. Vaccination has controlled the disease in Britain and Europe. Since a commercial vaccine could be made available in this country upon sufficient demand, the proper vaccination program could be used as a successful measure of control. Regulatory control measures seem unnecessary at this time.
Epididymitis of Rams

Epididymitis of rams, a specific infection caused by an as yet unclassified organism, has been recognized in California, Colorado, New Mexico and Oregon. New Zealand workers described this organism as *Brucella ovis* and the disease as brucellosis of sheep. American authors, however, are not in agreement with this classification. In the United States the limited studies conducted to date indicate that this disease does impair the fertility of the ram, but specific figures on its over-all economic importance are lacking. In the central valley of California it is common for 25 percent of the rams to be infected with this disease. In these valleys there is definitely a problem of fertility as regards sheep production. Initially it was thought that epididymitis per se could account for a big proportion of this infertility. However, subsequently it was found that sheep breeding operations in the mountain country where epididymitis is also prominent did not have the same infertility problem. It can perhaps be said that epididymitis combined with hot summer temperatures will reduce the fertility of a sufficient number of rams to account for infertility problems of economic importance. The inter-relationship of these two factors are under study.

To date the United States has not reported any serious consequences of this infection in the ewe. However, again referring to New Zealand, it is there reported that this organism does produce abortion, and is of major concern there. American workers should keep this in mind and search for it wherever possible. Recent work in California indicates that some rams not evidencing physical abnormalities may be infected with this organism and are detected only through the complement-fixation test. Despite this, it is felt that the clinically discernible disease should be grounds for eliminating interstate shipment of animals so affected.

This Committee recommends that veterinarians and breeders should be alerted to the existence of this specific epididymitis, and that it should be considered in certifying rams as healthy.

Scabies

The Committee is taking the liberty of invading the area of parasitic diseases by calling attention to the two transmissible diseases of sheep caused by scab mites and lice. Referring first to scabies, the prevalence of this disease of sheep in the central states continues to be a matter of grave concern to the large sheep-producing range areas of the west, where scabies was eradicated many years ago. In 1954 this Association passed a resolution requesting the Secretary of Agriculture “to take a firm stand to effect a complete eradication of scabies immediately.” In 1955 the Department of Agriculture announced that it had developed a tentative program for the eradication of sheep scabies. But a report issued by the Animal Disease Eradication Division indicates that while in 1954 scabies was reported in 391 flocks from 183 counties in 21 states; in 1959 the disease has been diagnosed in 736 flocks from 276 counties in 24 states. This would indicate that unless the control and eradication of the disease is greatly extended at both the state and federal levels the fight against scabies is lost.
In April, 1959, the National Woolgrowers Association called on the Secretary of Agriculture to “use all resources at his command to immediately begin a program that will bring about complete eradication of sheep scabies from the nation.”

On August 14, 1959, the California Wool Growers Association passed a resolution in which it “strongly urges the Committee on Infectious Diseases of Sheep and Goats and the Committee on Parasitic Diseases of the United States Livestock Sanitary Association to stress action leading to the eradication of scabies from this country.”

That sheep scabies can be eradicated in the central states is shown by the fact that on July 18, 1957, Mississippi and Louisiana were declared free of sheep scabies, as the result of a long time eradication effort by the states and United States Department of Agriculture.

Your Committee recommends that this Association go on record as strongly urging all states known to be harboring scabies infested sheep to redouble their efforts looking toward complete eradication of the disease, in a cooperative effort with the United States Department of Agriculture; and that this Association urge the enactment of necessary state legislation where laws are now known to be inadequate; and that it urge the appropriation of both federal and state funds needed to effect eradication.

A resolution on scabies eradication is being submitted to the Committee on Resolutions, advocating the establishing of a national federal-state scabies eradication project.

LICE

Heretofore sheep and goat lice were not considered to come within the purview of livestock regulatory authorities of either the state or federal government. It is becoming more evident that these parasites are assuming greater importance, causing unthriftyness and destroying wool and mohair. There are three species infesting sheep and several on goats. The sheep species are the biting body louse, Bovicola ovis, the sucking louse, Linognathus africanus, and the foot louse, Linognathus pedalis.

Your Committee believes that the increasing prevalence of sheep and goat lice warrants the recommendation that consideration be given by the state regulatory officials to pediculosis as a control problem. Some states now require that sheep moved into those states be certified as free from lice. In Montana sheep flocks that are found to be lousy are quarantined and dipped; and in Australia sheep that are moved from their home properties are inspected and if found lousy they must be dipped.
OUR PRESENT KNOWLEDGE OF RESERVOIRS AND VECTORS OF HOG CHOLERA VIRUS *

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The history of the spread of hog cholera to practically all of the areas involved in international commercial activity throughout the world indicates the importance of factors responsible for the rapid dissemination of this disease. According to Hutyra (1) hog cholera is supposed to have first appeared in North America in the State of Ohio in 1833 and spread rapidly over the entire populated area of this country. It was probably carried to Europe, as the disease was first recorded in England in 1862 and from England was first probably spread to Sweden in 1887 and to Denmark later that year as the result of an importation of infected animals. At the same time hog cholera appeared in southern France supposedly introduced from Algeria, spreading throughout France as well as Spain and Italy. Germany recorded a severe outbreak in 1893 which spread throughout the country and neighboring provinces of Austria. By 1895, hog cholera had reached parts of Hungary, Russia and Roumania. Since that time various regions in Asia, Africa, South America and remote areas in the Pacific as the result of military operations in the far east, have reported the incidence of hog cholera where ever swine husbandry has been developed. The spread of a potent virus infection from a focal point to universal proportions in comparatively few years indicates the existence of reservoirs and vectors capable of maintaining active virus for long periods of time under wide variations of climatic conditions. It is even more significant when the comparatively slow methods of transportation by boat and rail were taken into consideration which were the common means of transportation during the close of the last century. Attempts to secure reliable information on possible reservoirs and vectors of hog cholera virus as well as modes of transmission and effective means of disease control are not new. The earliest studies on hog cholera were directed toward the control and possible eradication of the disease. Extensive investigations were made to transmit the disease to man and domesticated animals as well as rats, mice and other rodents which commonly come in contact with diseased swine. Gerlach (2) in 1875 reported that hog cholera was communicable to man. Detmers (3) in 1880 described what he believed to be human infections in an Illinois family and the transmission of hog cholera to cattle. James Law (4) reported experimental transmission of hog cholera to lambs, sheep, rabbits and rats.

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Zichis (5) reported that hog cholera could be transmitted to sheep both by contact and experimental inoculations and he concluded that sheep could be inapparently affected with hog cholera and that they may be possible carriers. He also cited the receptivity of other animals to the action of hog cholera virus namely goats, rabbits and guinea pigs. Ten Broeck (6) studied the survival of hog cholera virus in laboratory animals especially the rat which could be a potential reservoir of the virus. Even the idea of dissociation of pathologic entities into inapparent forms and later being reactivated into highly pathogenic agents was published by Li (7) more than 30 years ago. Doyle (8) tested the survival of hog cholera virus in the bone marrow, muscle and skin of cured or processed hog carcasses. The duration of the activity of hog cholera virus in the feces and urine was studied by Geiger (9). Hog cholera virus was believed to exist in an inapparent form in man under experimental conditions by Nicolle and Balozet (10).

South African studies on the relationship of the viruses of the native swine fever to the European form of the disease by De Kock (11) and his associates indicated that recovered pigs carried active virus for as long as 10 months, and blood from apparently healthy wild wart hogs frequently produced hog cholera in domestic swine. The European and American types of hog cholera were introduced to South Africa about 1900 and the wild wart hogs were found to be reservoirs of infection. Jacotot (12) reported that hog cholera could be transmitted to sheep and goats by inoculation as well as simple cohabitation with affected swine; also that the disease in small ruminants was usually inapparent but the active virus may be discharged in the excrement. Birch (13) found that infected pork was a reservoir of active hog cholera virus. Active hog cholera virus was found to exist in simultaneously vaccinated swine for varying lengths of time following vaccination by McBryde (14) and he cites the contention of Michalka that the virus persists in the lymphatic glands as late as 10 months after vaccination which might be regarded as "latent reactions" and "virus carriers." The common hog louse (Hematopinus suis) was found to be a vector of hog cholera virus by Todoroff (15) about 25 years ago. Desiccation was found to be effective for the preservation of hog cholera virus by Munce and Reichel (16). Today, lyophilization which is simply desiccation under vacuum is the method of choice for the preservation of viral agents. The aerosol properties of lyophilized hog cholera virus were described by Schwarte and Mathews (17) making the spread of this disease probable through the action of winds and air currents.

The extensive review of the investigations on the transmission of hog cholera by McBryde (18) indicates several possible reservoirs and vectors of hog cholera. Infected hogs, carcasses of infected hogs either buried or unburied proved to be reservoirs of active virus throughout the winter and the colder periods in the spring. Warm and hot weather which is conducive to putrefaction rapidly destroys the virus. Cured and pickled pork products containing active virus were capable of reinfecting susceptible swine for a considerable period of time. Pigeons and rats should not be considered to be significant in the spread of the disease. Both the house fly and the biting stable fly were considered to be reservoirs and vectors of hog cholera virus.
KNOWLEDGE OF RESERVOIRS AND VECTORS

Some years ago the Canadian Division of Animal Pathology found that one particular area in Canada was subjected to more frequent outbreaks of hog cholera than the rest of the country. A survey in the area yielded information which directed suspicion toward the thorny-headed worm which seemed quite prevalent in the feeder hogs at that time. The report of Swales and Gwatkin in 1948 indicated that they had failed to demonstrate that this parasite was a reservoir or vector of hog cholera virus.

One of the greatest contributions to our knowledge of reservoirs and vectors of hog cholera virus was made by Shope (19, 20) during the past few years. His investigations indicate that the swine lungworm may serve as a reservoir and intermediate host for the hog cholera virus. Under ordinary conditions the virus is maintained in a masked or occult form and must be incited or activated to pathogenicity by some stress factor before the disease can develop in the infected swine. Shope found that the ascaris larvae provided the stress factor needed to activate hog cholera in swine infested with lungworms carrying masked hog cholera virus. The idea of the existence of hog cholera in an inapparent form was reported by Nicolle and associates (10) in 1932. Zichis (5) indicated that hog cholera could exist in an inapparent form in some of our domesticated animals. Shope (21, 22, 23) reported similar studies with swine influenza virus in which the swine lungworm acted as a reservoir and intermediate host for the virus in an inactivated form only to be provoked into pathogenicity by adverse weather as a stress factor. Extensive studies on the reservoirs and vectors of hog cholera virus have been carried on at the Veterinary Medical Research Institute at Ames for about 15 years as a part of the hog cholera program sponsored and supported by the Associated Veterinary Laboratories Inc. It was found that a limited number of immune swine vaccinated by either the simultaneous method using live virus and antiserum, or the modified living virus with antiserum developed a virus "carrier state" for periods up to 22 months (24). The virus was obtained from the blood stream periodically. The organs or tissues acting as reservoirs were not determined. The early studies were made on various birds, insects, external parasites and rodents in the area that came in contact with swine or had access to land, buildings and equipment used in swine production. Later on it was extended to reptiles and wildlife species. The birds included sparrows, robins, blackbirds, grackles, crows, hawks, owls, ducks, geese, and chickens. Various flies and mosquitoes constituted the insects studied. The reptiles included bull snakes, garter snakes and turtles. No hog cholera virus was secured from tissues of individuals of these groups and therefore it appears that these species do not act as reservoirs for hog cholera virus. Occasional transmission by biting insects and parasites can be accomplished if susceptible swine are in close proximity of cholera sick swine and constitute potential vectors but under normal farm conditions it is doubtful whether they constitute serious problems in the spread of the disease. Various birds coming in contact with cholera infected swine may be potential vectors but under field conditions have not proven to constitute serious problems as vectors of the infection.
Various forms of wildlife included rats, mice, woodchucks, opossums, raccoons, coyotes, ground squirrels, pocket gophers, rabbits and foxes. They were secured from their natural environment independent of their relationship to swine producing areas.

There was no indication that any of these wildlife species were reservoirs of hog cholera virus except two raccoons of many which were processed. The bone marrow preparation from one raccoon when injected into a susceptible test pig showed no significant temperature reactions or clinical manifestations for a period of two weeks. The experimental pig was then subjected to a stress factor by creating severe shock following the intravenous injection of heated swine serum. This method of creating a severe shock reaction in pigs was reported by Mathews and Buthala (25). Significant temperature elevation followed in 24 hours after the stress was applied. The temperature elevation reached a peak of 107.6 on the fifth day followed by a sharp temperature drop on the sixth day at which time the experimental pig died. Clinical manifestations which we associate with hog cholera developed rapidly after the second day. The infected animal was no longer able to stand after the fourth day. Post-mortem examination revealed well developed lesions characteristic of hog cholera. Filtrates from tissue emulsions made from the organs of this pig produced typical hog cholera infection in other test pigs.

The bone marrow filtrate from this raccoon apparently harbored the hog cholera virus in an inert or occult form and showed no untoward reaction when injected into a susceptible test pig. This virus was activated to pathogenic level by a severe stress factor in the form of shock. The animal developed typical hog cholera and a rather highly pathogenic strain of virus was secured from this experimental pig.

The second raccoon showed a somewhat different reaction. The bone marrow filtrate when injected into a susceptible test pig showed no significant temperature reaction for two weeks. The stress factor in form of shock was applied to the animal. Following recovery from shock the pig showed no unfavorable reaction for two weeks and then it was again subjected to the same stress factor. No unfavorable reaction was observed following recovery from shock. Two weeks following the second shock treatment the pig was challenged with 3cc of virulent hog cholera virus as an immunity test. The pig proved to be immune. It is difficult to evaluate these experimental results. They only illustrate another of the many peculiarities which have been associated with hog cholera virus for many years.

DISCUSSION

Much of the early research was directed toward a better understanding of the characteristics of hog cholera virus, the vectors and reservoirs, modes of transmission as well as the resistance of the virus to various environmental conditions. Much of this work especially involving man and other species of wild and domesticated animals has never been confirmed. Several of the early scientists believed that hog cholera virus could exist under certain
conditions in an inapparent form but scientific proof was never established. Shope produced evidence that certain internal parasites acted as reservoirs and vectors of hog cholera virus. Furthermore, the virus could exist in the host in an occult form which could be activated by certain stress factors. Shope used ascaris larvae as the stress factor. At least one species of wildlife was found to harbor the virus in a masked or inactive form that could be activated to pathogenicity by a stress factor which in this instance was severe shock. Other factors such as inclement weather, malnutrition or any condition which would reduce the body resistance sufficiently, could serve as a suitable stress factor necessary to activate the virus. The results of these investigations indicate that perhaps other reservoirs and vectors of hog cholera virus may exist and that the earlier work which lacks confirmation should be reinvestigated.

SUMMARY

The early studies on the transmission of hog cholera and the persistence of the activity of the virus under natural conditions furnished us with much basic information on possible reservoirs and vectors of hog cholera virus.

The vectors of hog cholera virus incriminated in the transmission of hog cholera include insects, parasites, infected meat, and meat products, infected carcasses, feeds, pens, buildings and equipment. Trucks, railroad equipment, stockyards and man as well, have contributed to the spread of the infection. Subsequent studies indicated that infected swine carcasses whether buried or unburied served as reservoirs of active virus for months in the cooler seasons of the year. The summer months provided conditions highly favorable for putrefaction which apparently is the greatest natural agent conducive to the destruction of the virus. Preserved and smoked pork containing active virus may be a reservoir of infection. Certain parasites may act as reservoirs and intermediate hosts for the virus. At least one wildlife species has been found capable of acting as a reservoir of hog cholera virus.

Positive experimental evidence that the virus may exist in a masked or inactive form which under certain conditions can be activated to pathogenicity by stress factors contributes much to our knowledge of hog cholera virus. Much of the early research and clinical observations which lack confirmation should be reinvestigated in view of our present knowledge.

The questions raised regarding many of our field problems may soon be answered when more basic information on hog cholera virus is available. Postvaccination problems and failure to develop a satisfactory immunity under field conditions may be better understood when more is known about the wide variation in the characteristics of the different virus strains. The reservoirs and vectors of hog cholera virus should be given careful consideration in any effective program designed for the control and possible eradication of hog cholera.
REFERENCES

24. Unpublished data from the Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


All members of the Committee on Transmissible Diseases of Swine present at meeting, with the exception of Dr. J. D. Ray, have met and discussed the problems of this organization that fall within the scope of this Committee. Doctor Ray was absent due to pressing matters before other committees to which he was assigned.

We wish to thank Dr. F. J. Mulhern for meeting with the Committee and lending his knowledge and advice to us in our deliberations. Doctor Mulhern has recently attended a meeting of the swine industries conference and presented to us a proposal from that group that a national committee representing all facets of swine disease research, various segments of the swine industry, the United States Department of Agriculture and the United States Livestock Sanitary Association be set up to appraise all swine disease research being conducted in this country, to set up priorities on these research programs and to recommend disease control programs. We strongly urge that this Association lend its support to the formation of such a committee.

This Committee is aware of the critical need for funds to conduct swine disease control programs throughout the country. We urge this Association to continue to insist that a swine disease section be set up in the animal disease eradication branch of the agricultural research service and that sufficient funds be made available so that swine disease control programs may be conducted in cooperation with the various states.

For several years this Association has had a committee on transmissible diseases of swine and a committee on nationwide eradication of hog cholera. Little has been accomplished toward beginning any disease control programs in swine. We can see no further need for two committees and suggest that these committees be combined and strengthened. The new committee should be charged with the responsibility of making a survey of all swine diseases and setting up a list of priorities for disease control programs. We suggest further that this Committee make a study of all swine disease research programs being conducted at the present time and to recommend to this Association the necessary action to strengthen and coordinate these research programs.
PROGRESS REPORT OF THE EXPERIMENT ON ERADICATION OF
HOG CHOLERA IN THE FLORIDA PILOT TEST AREA—
FISCAL YEAR 1959

M. R. ZINOBER, D.V.M., and SEIBERT L. BERLIN, V.M.D.*

Live Oak, Florida

The designation of Suwannee County, Florida, as a hog cholera pilot test eradication area, and the establishment of a Hog Cholera Research Station by the United States Department of Agriculture, Animal Disease and Parasite Research Division and the Florida Livestock Board were reported to this Association in 1958.1 During fiscal year 1959, the Hog Cholera Research Station continued investigations in two general areas to determine (1) the immunogenic efficiency of all types of modified live virus vaccines administered with a minimum dose of 15 ml. of hog cholera antiserum, and (2) the status of eradication of the disease from the pilot test area by attempting to establish the incidence of hog cholera in the area. In the fiscal year ending June 30, 1959, 32,934 swine in 1,104 herds have been vaccinated. Since there are approximately 60,000 swine on 1,500 farms in Suwannee County, these figures represent vaccination of 54.9 percent of the swine in 73.6 percent of the herds in the county. Table 1 gives the breakdown of these data according to the type of vaccine used.

<table>
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<tr>
<th>Vaccine</th>
<th>Herds No.</th>
<th>Percentage of Total</th>
<th>Pigs No.</th>
<th>Percentage of Total</th>
<th>Average Serum Dose</th>
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<tr>
<td>Lapine Origin</td>
<td>421</td>
<td>38.1</td>
<td>13,239</td>
<td>40.2</td>
<td>17.4</td>
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<tr>
<td>Porcine Origin</td>
<td>277</td>
<td>25.1</td>
<td>9,439</td>
<td>28.7</td>
<td>15.0</td>
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<tr>
<td>Tissue Culture</td>
<td>406</td>
<td>36.8</td>
<td>10,256</td>
<td>31.1</td>
<td>19.5</td>
</tr>
<tr>
<td>Total</td>
<td>1,104</td>
<td>100.0</td>
<td>32,934</td>
<td>100.0</td>
<td>17.4</td>
</tr>
</tbody>
</table>

Also in fiscal year 1959, 640 vaccinated hogs in 327 herds have been challenged. The hogs were challenged with 1 ml. of Station virus s.n. 1, according to the method and procedure described in our report to this Association in 1958. The latest titration of the challenge virus, which was completed on September 4, 1959, demonstrated that it had a minimum lethal dose of $2 \times 10^{-6}$ ml. Table 2 gives the challenge results for fiscal year 1959 and the cumulative results since commencement of the work in April, 1957.

* Florida Hog Cholera Research Station, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Live Oak, Florida.

TABLE 2

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Fiscal Year 1959 Adequately Protected</th>
<th>Cumulative Since April, 1957 Adequately Protected</th>
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<tbody>
<tr>
<td></td>
<td>Herd* Number* Percent</td>
<td>Number* Percent</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numerator = Number adequately protected. Denominator = Number challenged.

Although the data in Table 2 seem impressive, they represent a marked and significant decline in the percentage of protection from previous years. This decline is shown in Table 3.

TABLE 3

Variation in Percentages of Protection of Pigs Vaccinated With Modified Live Virus Vaccines During Fiscal Years 1957, 1958 and 1959

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Fiscal Year 1957 Number* Percent</th>
<th>Fiscal Year 1958 Number* Percent</th>
<th>Fiscal Year 1959 Number* Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Lapine Origin</td>
<td>65 97.0</td>
<td>389 93.7</td>
<td>137 81.5</td>
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<tr>
<td></td>
<td>67 97.0</td>
<td>415 93.7</td>
<td>168</td>
</tr>
<tr>
<td>Porcine Origin</td>
<td>79 95.2</td>
<td>377 97.2</td>
<td>176 81.9</td>
</tr>
<tr>
<td></td>
<td>83 95.2</td>
<td>388 97.2</td>
<td>215</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>46 97.9</td>
<td>422 93.8</td>
<td>223 86.8</td>
</tr>
<tr>
<td></td>
<td>47 97.9</td>
<td>450 93.8</td>
<td>257</td>
</tr>
<tr>
<td>Total</td>
<td>190 96.4</td>
<td>1,188 94.3</td>
<td>536 83.8</td>
</tr>
<tr>
<td></td>
<td>197 96.4</td>
<td>1,253 94.3</td>
<td>640</td>
</tr>
</tbody>
</table>

* Numerator = Number adequately protected. Denominator = Number challenged.

The question of the cause of this decrease has been considered from the standpoint of variation in the defense mechanism of the pig, variation in immunogenicity of the vaccine or in the potency of the serum, or a combination of all. Experiments are now in progress on several of the facets of the problem and cannot be reported on at the present time. However, a few general observations can be made.

In order to study the role of the pig as a contributory factor in the decline of the percentage of protection, consideration is being given to the significant
changes in inbreeding practices in recent years. These practices suggest the possibility that a swine population is being produced in this country similar to Canadian swine, which are generally considered to be difficult to immunize. In a further consideration of the role of the pig in the decline of the percentage of protection, correlations were attempted between the number of days of post-vaccination challenge and the variation in the percentage of protection. In an examination of the data, it was found that in pigs which were vaccinated with lapine origin vaccine and were inadequately protected, the average number of days post-vaccination on which they were challenged was 247.0 days whereas in pigs which were adequately protected, this figure was 352.4 days—a difference of 105.4 days. In other words, more pigs, vaccinated with lapine origin vaccine, were adequately protected when challenged later after vaccination than when challenged earlier. In pigs which were vaccinated with porcine origin vaccine and inadequately protected, the average number of days post-vaccination was 170.7 days and for adequately protected pigs this figure was 217.2 days, a difference of 46.5 days. Here, again, more pigs were adequately protected when they were challenged later after vaccination than when they were challenged earlier. These figures seemed to suggest that the greater the number of days post-vaccination, the higher the percentage of protection. In pigs vaccinated with tissue culture vaccine, there seemed to be no significant difference between the number of days post-vaccination of adequately protected pigs (170.8 days) and inadequately protected pigs (180.0 days).

In an examination of all the data available, including the challenge results of tissue culture-vaccinated pigs, it was found that during the first 12 months of the collection of data—that is, from April, 1957, to April, 1958, inclusive—the percentage of protection in pigs was 95.3 percent and the average number of days post-vaccination on which they were challenged was 367.8 days. In contrast to this, during the next 14 months—that is, from May, 1958, to June, 1959, inclusive, which, incidentally, includes the fiscal year covered by this report—the comparable figures were 85.0 percent of protection and 185.7 post-vaccination days. In other words, the hogs were being challenged 182.1 days earlier and their percentage of protection was about 10 percent lower. Thus, the decrease in percentage of protection which has been noted previously during fiscal year 1959 seems to have been associated with challenges at the end of a shorter post-vaccination period than in previous fiscal years.

The positive determination of the presence or absence of hog cholera virus on the farm, obviously, is a prime requisite in achieving progress toward effective control.

Specimens for test were submitted by the local practicing veterinarians or by a swine-raiser at the request of his own veterinarian if hog cholera were suspected, even remotely. The veterinarians were provided with sterile citrate test tubes and mason jars for the collection of blood or other tissues at necropsy. The owners were usually requested to bring a live pig to the Hog Cholera Research Station where station personnel performed the necropsy and collected the specimens. At that time, a detailed history of the case was
taken. The questioning elicited—in addition to the usual breeding, nutrition, and sanitation information—a detailed history of purchases including dates, numbers of swine, places of purchase, vaccination of purchased swine and date of vaccination, and name and address of the veterinarian. Questions were also asked regarding vaccination of the home herd. In regard to sickness and losses, particular attention was paid to whether the suspected disease had occurred in the vaccinated purchased swine or in the vaccinated or nonvaccinated home herd, and whether the specimen was from a vaccinated or nonvaccinated pig. The serial numbers of the vaccine and antiserum used in the home herd were indicated by the veterinarian by means of ear notching according to a pre-arranged system. With regard to the outbreak itself, information was requested as to the date of the first onset of disease, number of swine involved, the number that were sick, and the number that had died. The signs of sickness and the findings at necropsy were also requested. After the procurement of suitable specimens, bacteriological cultures were made, and the specimen was prepared for injection. If the specimen was citrated blood it was ready for injection immediately. If the specimen was another tissue such as spleen, lymph nodes, or similar tissues, it was macerated in sterile sand and sterile normal saline solution, centrifuged, and the supernatant was collected. This represented a suspension of the tissue specimen and was used for injection.

The test itself was made with two susceptible pigs and one hog cholera-immune hog. The susceptible pigs were procured from non-vaccinated herds outside of Suwannee County if the owner had agreed to identify the litters. Upon delivery to the station, the pigs were held in isolation for 30 days and then one pig from each litter was injected with 1 ml. of a known virulent virus to demonstrate the susceptibility of the litter. Two pigs from the same litter were used to test any one specimen. The cholera-immune hog was a vaccinated animal which survived challenge with virulent virus.

The anti-hog cholera serum used in the test was a stock commercial product. It was titrated after purchase, but before use, to determine the minimum protective dosage and the duration of immunity. As a result of this titration it was demonstrated that pigs injected with 40 ml. of the antiserum were adequately protected against 1 ml. of virulent hog cholera virus up to and including the twenty-first day after injection but were susceptible again at the twenty-eighth day.

The test itself consisted of injecting one susceptible pig with 5 ml. of the specimen suspension only (S). The other susceptible pig was injected with 5 ml. of the specimen suspension simultaneously with 0.5 ml. per pound of live weight of the previously titrated stock antiserum (S and S). The cholera-immune hog (CI) was injected with 5 ml. of specimen suspension only. All of the pigs were penned together and were observed twice daily.

On the seventh day, a small quantity of blood was taken from the S pig and was stored for future serial passage.

A reaction was considered positive if (1) the S pig showed signs of hog cholera during the course of the disease and characteristic lesions at necropsy, (2) the S and S pig remained well and, when injected with 1 ml. of virulent
ZINOBER AND BERLIN

hog cholera virus at 28+ days, also remained well, and (3) the CI hog remained well for 14+ days.

A reaction was considered negative if (1) the S pig remained well for 21+ days and, when injected with 1 ml. of virulent hog cholera virus, showed signs of hog cholera during the course of the disease and characteristic lesions at necropsy, (2) the S and S pig remained well for 28+ days and when injected with 1 ml. of virulent hog cholera virus showed signs of hog cholera during the course of the disease and characteristic lesions at necropsy, and (3) the CI hog remained well for 14+ days. In one case, an immunizing virus was demonstrated in the specimen submitted. In this case, all of the test pigs remained well. When the S pig and the S and S pig were injected with 1 ml. of virulent hog cholera virus at 21 and 33 days respectively, they also remained well.

During fiscal year 1959, 23 specimens from as many suspected herd-cases of hog cholera were submitted for test. As the result of animal inoculation tests as described above, eight of the 23 specimens were found to be positive; the swine in six of the eight herds had been vaccinated and those in the other two herds had not been vaccinated. Upon subsequent investigation, it was disclosed that, in each of the herds with positive cases, there was a recent addition from a public market. It is interesting to note that in the first four months of the current fiscal year, 28 additional specimens have been submitted. Of the 28 specimens, there were nine positive, which is more than in all of fiscal year 1959. Of these nine positive cases, three were from non-vaccinated swine and six from vaccinated swine. All but one of the nine positive specimens were associated with recent purchases at a public market. The one case that was not associated with a public market purchase was already infected before the swine were vaccinated. The owner had destroyed all of the swine showing signs of cholera and called the veterinarian to vaccinate the remainder of the herd. This was done and those swine that were in the incubative stage of the disease subsequently developed a fulminating form of hog cholera.

The locations of each of the 17 positive herds have been pinpointed on a large aerial photographic map to determine if their geographical sites were related to the possibility of dissemination from another herd. This was found not to be the case. All 17 cases were found to be well scattered through the county, the closest being several miles apart. After personal visits to each of the 17, it was found that no unusual contact occurred between or among the relatively few farms involved. The only thing in common was, as mentioned previously, recent purchases of swine at a public market.

As reported last year, the Florida Livestock Board passed a resolution, effective July 1, 1958, making it mandatory that all non-vaccinated swine passing through any public market in Suwannee County and not intended for immediate slaughter be vaccinated before being removed from the market premises. Although, theoretically, this is a good resolution and has been observed to the letter by the honest and well-meaning swine-raiser, it has merely served as a temporary obstacle for the unscrupulous swine-raiser. To paraphrase an old saying, "Locks and laws are only made for honest
ERADICATION OF HOG CHOLERA IN FLORIDA

people.” Our laws and regulations for animal disease control are gladly received by the honest and well-meaning swine-raiser for it indicates to him what must be done to control the spread of animal disease. The unscrupulous swine-raiser, on the other hand, merely looks for loopholes and usually finds them in any regulation which interferes with his operation. He usually tries to stay within the law, but his hesitation would only be momentary if he had to step outside the law. The unscrupulous swine-raiser operates in somewhat the following manner. He buys nonvaccinated feeder swine. If an outbreak of hog cholera occurs, he destroys the sick animals, calls upon a veterinarian to vaccinate the seemingly well pigs, and then sells them immediately at a public market. The swine, although vaccinated, are in the incubative stage of the disease. When these swine are brought back to the purchaser’s farm, there is an outbreak of hog cholera in a few days in what seems like vaccinated swine. As mentioned before, there is no law that an unscrupulous operator cannot circumvent, but it may be possible to make this circumvention somewhat more difficult. A simple procedure might be to require that all swine sold at a public market be accompanied by a certificate showing vaccination not less than 30 days prior to the sale.

Although variations in pigs and variations in vaccines may influence the success or failure of an animal disease program, the public market still seems to be the weakest link in the control program in our area. It is the focus from which hog cholera can be carried back to Suwannee County farms. The public market is an institution with which we will have to live but there is no reason why we cannot attempt to legislate it into conformity with a fair and rational hog cholera control program. As described above, the Florida Livestock Board has attempted to correct this situation by regulation, but it is believed that more specific laws with more severe penalties are needed.

The incidence of hog cholera in the pilot eradication test area is only a partial indication of progress in control of the disease. It is obvious, that if the incidence of the disease is low in surrounding areas and in the nation as a whole, a determination of the effectiveness of control measures cannot properly be made with complete assurance. Low incidence of hog cholera in the area may merely signify insufficient exposure. The final test will come when the incidence of hog cholera in the contiguous counties is markedly higher than in Suwannee and when the pilot test eradication area remains as a cholera-free island surrounded by a sea of infection.
REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


Your Committee has heard and considered detailed reports which have been presented concerning hog cholera outbreaks in some of the mid-western states during the past summer. These reports demonstrate that the number of cases and resultant losses were of far less magnitude than had been rumored, and further, that the majority of laboratory-confirmed cases occurred in non-vaccinated swine rather than in modified virus-vaccinated herds as had been indicated. While it should be pointed out that some so-called breaks did occur in herds vaccinated with modified live virus, there were indications that some troubles not directly associated with cholera itself were responsible for serious losses in a few of these herds. Faulty handling of products and usage in poor risk herds were also incriminating factors in the reports considered.

From a thorough evaluation of the data presented on these outbreaks, your Committee is of the opinion that there is little justification for the incrimination of modified live virus vaccines in the failure to establish immunity to hog cholera; recognizing further that while no product presently available will adequately protect all animals vaccinated, there is no reason to believe that vaccination incorporating the use of modified live virus will not confer comparable immunity to that obtained through the use of virulent virus if sound judgment and proper techniques are employed in the use of these products.

Your Committee wishes to commend the 32 states to date which have outlawed the use of virulent virus, and re-emphasizes recommendations in reports of previous years that this policy be established on a nationwide basis.

The increased use of modified live virus vaccine throughout the nation and resultant reduction in serum reserves, concurrent with the decrease in swine vaccinations have set the stage for a tremendous economic loss in the event of a severe hog cholera outbreak. Your Committee is of the opinion that increased backlogs of serum should be maintained for use in the event of such an outbreak; however greater effort should be directed toward increased swine vaccination throughout the country.

Your Committee endorses the establishment of a Swine Disease Committee within the National Swine Industry Conference, one of whose aims is directed toward the implementation of hog cholera eradication programs in the several states. Your Committee further recommends when such a program
is established within a state that this action be reported to the United States Department of Agriculture, Animal Disease Eradication Division. The Committee calls attention to the 1956 Report setting forth the 10 cardinal measures necessary in establishing a sound program for the control and eradication of hog cholera.

Your Committee wishes to again emphasize the need for continued research in the improvement of immunizing agents and in the development of better diagnostic techniques in the detection of hog cholera.

The pamphlet "What One Should Know About Hog Cholera" is now in process of printing and will be available for distribution in early 1960. Your Committee wishes to express its appreciation to those who have contributed to the publication of this pamphlet.
TWO NEW IMMUNOLOGICAL TYPES OF VESICULAR EXANTHEMA VIRUS

A. A. HOLBROOK, D.V.M.; J. N. GELETA, D.M.V.; M. S. and S. R. HOPKINS, B. S.*

Beltsville, Maryland

The plurality of immunological types and the ease with which vesicular exanthema virus (VEV) mutates have complicated the diagnosis of this disease and have made the typing of the virus more difficult. Crawford (5) identified four types and called them A, B, C, and D, based on cross immunity tests with virus material collected from the California outbreaks in 1933 and 1934. The California workers (7) recovered three types from the 1940-42 outbreak and started a new series identified as types A, B, and C. Brooksby in 1954 (4) reported that the 1934 B isolate of Crawford and the 1943 101 strain collected by Traum were still virulent. All other isolates collected prior to 1948 have been lost.

A third series of VEV type collections was started in 1948 and has continued to the present time. These types are now identified alphabetically in the sequence in which the new types are confirmed followed by the year collected. The types in this series are as follows: A48 reported by Madin and Traum (8); and B51, C52, D53, E54, F55, G55, H54 and I55 reported by Bankowski et al. (1, 2, 3). Bankowski (2) reported that the types 1, 1934 B and 1943 101, isolates held in the Virus Institute Pirbright, England, are immunologically different from types A through I.

HISTORY

All field outbreaks of vesicular exanthema (VE) outside of California have been considered to be type B51 (1, 10). The first virus isolates outside of California to show irregular immunological characteristics were from three closely associated garbage-feeding establishments in Secaucus, New Jersey. The disease was found on these premises in 1952 and 1953 and was diagnosed by animal inoculation. The hogs were disposed of by processing as they attained market weight. There was no complete animal depopulation, and no virus samples were collected for typing.

The disease was found again in 1954 on these three premises and diagnosed by animal inoculation. Infected and exposed hogs were slaughtered, and the meat was processed. Complete animal depopulation again was not accomplished. Virus material from each of the three premises was collected and sent to the laboratory where the material was held in a dry ice chest.

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On August 3, 1956, the disease appeared on one of these premises and the isolate was designated as Sec. 1–56. It was found on the adjoining premise on October 30, 1956, and this isolate was designated as Sec. 2–56. Questioning of the owner revealed that the hogs which were infected had been purchased from the third premise. Infection was found November 8, 1956, on this third premise and the isolate was designated as Sec. 3–56. This is the last known VE field infection in the world. The premises were completely depopulated of swine, thoroughly cleaned and disinfected, and allowed to stand vacant for some time before restocking. Continued inspection revealed no evidence of the disease.

**MATERIALS AND METHODS**

Isolation Techniques.—To maintain strict isolation of the agents, all experiments were conducted in barns and laboratories specially designed and used solely for VE studies. Personnel entering the building were required to remove all street clothing and change into coveralls, boots, and rubber gloves. All material and clothing were sterilized or disinfected before removal from building, and all personnel were required to shower before leaving the quarantined areas. Every effort was made to confine the studies to one virus strain in the barns at one time. At the conclusion of each experiment all animals were killed within the unit and the carcasses were incinerated. The barns were thoroughly cleaned and sprayed with a four percent lye solution.

Hogs recovered from VE that were to be used for cross immunity studies were held in isolation for at least three weeks from the time of the last active viral infection. They were then removed to special holding units and susceptible swine were placed with them as controls to test for the presence of active virus. These controls were later challenged with virus of homologous type.

Virus.—Virus material of types A through I, obtained from Doctor Bankowski, and the 1954 and 1956 isolates from the three premises in Secaucus, New Jersey, was passaged in susceptible pigs and the vesicular coverings were harvested. This virus material was used as follows: (1) inoculated into other susceptible pigs to produce virus material, immune serum, and immune pigs for cross immunity studies, (2) inoculated into horses, cows, chickens, and guinea pigs to test for susceptibility of these animals; and (3) adapted to swine kidney tissue culture cells.

In all experiments appropriate controls were included to test for the activity of the virus. If any of the controls were negative, the experiment was repeated.

Cross Immunity Studies.—Immune swine were produced to each virus type and held separately under strict quarantine until used. Two pigs from each immune group were moved into pens within a barn. Each pig was inoculated on the coronary band and the interdigital space of the two front feet with a 10 percent suspension of the virus material to be studied. The pigs were observed for vesicles and any febrile response.
Serum Neutralization Studies.—Tissue cultures for serum neutralization were primary cultures of trypsinized swine kidney in stationary tubes as described by Hopkins (6). Growth was started in Hank's balanced salt solution and maintained in Earle's balanced salt solution. Both nutrient solutions contained five percent calf serum and 0.25 percent lactalbumin hydrolysate. One hundred units of penicillin and 100 micrograms of streptomycin per ml. were included. After six days incubation at 37°C. with one or two fluid changes, the cultures were ready for use.

For the serum neutralization test a 1:5 dilution of each serum sample was inactivated at 56°C. for 30 minutes. Twofold dilutions of the inactivated serum were made and 0.5 ml. was mixed with an equal amount of virus diluted to contain 500 ID$_{50}$/0.1 ml. After standing one hour at room temperature, 0.2 ml. of the virus-serum mixture was inoculated into each tissue culture tube. The tubes were allowed to stand upright for 10 minutes to insure close contact between cells and inoculum. The tubes were then placed in an inclined position and incubated at 37°C. Readings were made at 24 and 48 hours with the final reading at 72 hours. The 50 percent endpoint was calculated by the procedure of Reed and Muench (11) and expressed as the reciprocal of the dilution which would protect 50 percent of the cultures against 500 ID$_{50}$/0.1 ml. of virus or the medial protective dose (PD$_{50}$).

RESULTS

Animal Inoculations.—Horses were inoculated intradermally on the tongue with each type of VEV. Only those horses inoculated with type D53 virus developed vesicles. When vesicle coverings from these horses were ground and passed to other horses the reactions were less severe. In a horse inoculated with type A48 virus, necrosis was observed along the lines of inoculation. This necrotic material was harvested and passed to a second horse but failed to produce lesions. All horses inoculated with the remaining types of VEV were negative.

In serological studies with serums from convalescent and hyperimmune VE horses, complement fixing and neutralizing antibodies were detected. Significant titers were obtained with these serums against homologous antigens of swine tissue origin. Cross fixation was also observed with most heterologous antigens of the other VEV types. However, no one serum was found which had diagnostic titers against all types of VE virus and showed no fixation with vesicular stomatitis (VS) antigens.

Hyperimmune anti-VE horse serum types A48, C52, D53, E54 and J56 were tested for neutralizing antibodies by the tissue culture procedure and found to have diagnostic titers and to be type specific. The neutralizing antibody rose rapidly and reached its peak in about 11 days following the hyperimmunizing dose, which was administered intradermally three to four weeks after the primary exposure. Cows and adult chickens inoculated intradermally on the tongue and guinea pigs inoculated intradermally on the plantar pads with a 10 percent suspension of each virus type were negative.
ADAPTATION OF VIRUS TO TISSUE CULTURE

All the virus types A through I and the Secaucus 54 isolates adapted readily to swine kidney cells in tissue culture with cytopathogenic effect. The three Secaucus 56 isolates were very difficult to adapt to tissue culture. Sec. 1-56 and Sec. 3-56 adapted to tissue culture with consistent cytopathogenic effect only after some 15 alternate passages between pigs and tissue culture. No attempt was made to adapt Sec. 2-56 by the alternate passage technique.

Identification of K54 (Sec. 3-54) and J56 (Sec. 3-56).—The three 1954 Secaucus isolates which had been held for five years in a dry ice chest produced good vehicles with extensive lesions and secondary vesiculation when inoculated into pigs. All three of them adapted readily to tissue culture. When typing studies were conducted, Sec. 1-54 isolate proved to be type B51; Sec. 2-54 isolate proved to be type B51 with some cross neutralization with type J56. Sec. 3-54 isolate had a low level of cross neutralization with type C52 but was determined to be a new type and was identified as K54.

The three 1956 Secaucus isolates were of very low virulence. When the virus suspensions were filtered or treated with antibiotic to suppress contamination and inoculated intradermally into the coronary bands and the interdigital spaces of the feet of susceptible pigs, little or no febrile response was recorded. The vesicles were small (3 or 4 mm.) with little extension or diffusion. However, if bacterial contaminants were in the inoculum, a decided febrile response was recorded. Temperatures of 106.0°-108.0°F were common. There appeared to be a symbiotic relationship between the virus and the contaminants.

Serological studies by cross neutralization showed that these three 1956 Secaucus isolates were all of the same type and were identified as J56. When cross immunity studies were conducted in immune swine, differences were

<table>
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<tr>
<th>Challenge Virus Type</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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+ Immune to challenge.
– Susceptible to challenge.
ND Not done.
found. In most instances, immune swine of each isolate were susceptible when challenged with each of the other two isolates. However, the appearance of vesicles was delayed 24-48 hours beyond the appearance of vesicles in controls or in swine immune to the other types of VE. Therefore, on the basis of present information, the three strains of J56 are maintained.

Confirmation of Types A through K.—The cross immunity studies in swine are shown in Table I. Each of the 11 virus types was used to challenge immune swine of types A48 through G55 and J56. In addition, swine immune to H54 and I55 were challenged with K54 virus, and K54 immune swine were challenged with C52 virus. All the swine were immune to challenge with homologous type virus but were susceptible to challenge with the heterologous type viruses.

Further cross immunity studies with H54, I55, and K54 were interrupted by the announcement that VE has been eradicated, and a decision was made to terminate research on VE at Beltsville.

TABLE II
Results of Cross Neutralization Studies With Eleven Types of Vesicular Exanthema Virus

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2,560*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>5,120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>5,120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2,560</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>320</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>500</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>G</td>
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<td>0</td>
<td>320</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>900</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1,600</td>
<td>0</td>
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</tr>
<tr>
<td>J</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5,120</td>
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<tr>
<td>K</td>
<td>0</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>500</td>
</tr>
</tbody>
</table>

* PD₅₀ medial protective dose.

The results of the serum neutralization studies are shown in Table II. The neutralization titers of all the types of immune sera were of diagnostic levels with their homologous viruses. Low level neutralization occurred between types C52 and K54. In addition, there was slight neutralization between I55 hyperimmune serum and type G55 virus, but no neutralization was found between I55 virus and G55 serum. The neutralization titers of all the other types of immune sera with heterologous virus were negative or so low as to be of no importance.

DISCUSSION

Serum neutralization in tissue culture presents a rapid and accurate method of diagnosing and typing VE. The value of this technique is exemplified by its use in the identification of the new virus type K54. This virus type was established in a few weeks. The time was required chiefly to pre-
pare antiserum to this type. In all probability if VE does reappear, the virus will be of a different type and the savings in time and expense by use of this method will be obvious. The genetic instability of VEV is illustrated by the fact that in every year between 1951 and 1956, at least one new type—and in some years more than one type—has been identified (3, 9). Studies of the viruses from one premise in the Secaucus area support the concept of field mutation. Within a period of two years, two new types, K54 and J56, were recovered from this premise. It is reasonably assumed that one of the earlier outbreaks on this premise was type B51, as this type was diagnosed in that area at the time. The cross neutralization between types C52 and K54 is particularly interesting in view of the fact that each of these types was isolated at a time that type B51 was known to be causing infection in the areas from which they were isolated. This suggests the possibility that these two types are mutants of B51 and have common antigenic characteristics.

As a laboratory diagnostic technique, serum neutralization is capable of differentiating VE, foot-and-mouth disease (FMD), and VS. Each of the types of VEV (A through K) was tested against antiserum from swine immune to New Jersey and Indiana types VS and against guinea pig hyperimmune anti-FMD serums, types A, O, C and South African types one, two and three. In no instance was there any neutralization of VEV (unpublished data). Immune serums of known titers have been made available to the Animal Disease Eradication Division of the Agricultural Research Service for use in the differential diagnosis of vesicular conditions.

The older method of typing by cross immunity should not be completely discontinued but rather should be used in conjunction with the serum neutralization test. This is illustrated by the fact that cross neutralization placed the three isolates from the 1956 Secaucus outbreak in the same type; whereas cross immunity studies showed immunological differences between them. These differences were enough to justify the maintenance of these three isolates as separate strains. Further strains. Further study of these three strains would be desirable, especially the use of the plaque technique in tissue culture to evaluate the extent of these variations.

The possibility that virus from a future field outbreak would not readily infect cells of the tissue culture system, as was found with the J56 viruses, remains as a deficiency of the neutralization test. A virus adapted to produce dependable cytopathogenic effect in the cultured cell is a prime requisite for this test. It is of interest that although the type J56 viruses were of low pathogenicity and did not adapt readily to tissue culture cells, the neutralizing antibody titers were as high as some of the more virulent types.

In an effort to find a serum that would contain antibodies to antigens common to all types of VEV, several different trials were made (unpublished data). In one of these trials, swine were inoculated with a mixture of virus types A through J. The antiserums from these swine neutralized the viruses of the types inoculated, but they did not neutralize type K54 virus which was not used in the inoculum.

Early work with hyperimmune horse serums led to the conclusion that the serums of one type of VE gave sufficient complement-fixation titers with the
other types of VE to differentiate VE from VS. However, in a continuation of these studies with all 11 types of VEV, it was found that the titers of some of the VE types were of the same level as the VS titers.

In a search for other means of differential diagnosis between VS and VE tissue culture, cells taken from monkeys, bovines, mice, swine, and seven different cell lines of human origin were inoculated with vesicular stomatitis virus (VSV) and type B51 VE virus. VSV produced cytopathogenic effect in all of the cell systems, whereas VEV produced a cytopathogenic effect only in the cells of swine origin.

Following the announcement on October 22, 1959, by Secretary of Agriculture Benson that VE had been eradicated from the United States, studies with VEV were discontinued at Beltsville. All VE material including the 11 virus types, pools of immune serums of each type, and the collection of field samples were moved to the United States Department of Agriculture’s Plum Island Animal Disease Laboratory for use in diagnostic and research studies. All facilities and equipment used in VEV studies were thoroughly cleaned and disinfected twice, and allowed to remain idle for varying lengths of time before being used for other disease studies.

SUMMARY AND CONCLUSIONS

1. Two new immunological types of vesicular exanthema virus were isolated from hogs on a garbage feeding establishment in Secaucus, New Jersey. The 10th and 11th types were identified as J56 and K54, respectively.

2. Type J56 is characterized by low pathogenicity for swine and difficult adaptation to tissue culture cells, whereas K54 is characterized by high pathogenicity for swine and easy adaptation to tissue culture cells.

3. VEV types A through K were confirmed as immunological types by cross neutralization studies in tissue culture and cross immunity studies in swine.

4. Horses were inoculated with each type of VEV. Only those horses inoculated with type D53 developed vesicles. In a horse inoculated with type A48, necrosis was observed along the lines of inoculation. All horses inoculated with the remaining types of VEV were negative.

5. Cows, adult chickens, and guinea pigs were negative when exposed to all 11 virus types.

6. The diagnosis and the typing of VEV are discussed.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the veterinarians and livestock inspectors of the State of New Jersey and the United States Department of Agriculture for their efforts in collecting and submitting field samples and data which made this study possible.

Sincere appreciation is extended to Drs. L. O. Mott, O. L. Osteen, and W. C. Patterson of the Animal Disease and Parasite Research Division for their valuable advice and help in these studies.

The assistance of Dr. E. W. Jenney of the Animal Disease Eradication Division and Mr. Henry Tribble is gratefully acknowledged.
REFERENCES


THE INFECTIVITY ASSAY OF FOOT-AND-MOUTH DISEASE VIRUS IN SWINE

J. H. Graves, D.V.M. and H. R. Cunliffe, D.V.M.*

The study of disease depends in part upon methods available for the assay of the infectivity of the infectious agent concerned. The activity of a virus in the definitive host is not necessarily reflected by the activity of that same virus in tissue culture or small experimental animals. Methods for the evaluation of virus infectivity in each of the naturally susceptible species are necessary in the study of diseases such as foot-and-mouth disease (FMD). The technique of titrating foot-and-mouth disease virus (FMDV) by inoculating the bovine tongue as described by Henderson has been of considerable value in the quantitative study of the disease in cattle (1).

The importance of swine in the livestock economy of the United States as well as their association with cattle in many farming practices has led to increased study of experimental FMD in that species. Attempts to develop a titration technique in swine similar to the tongue titration of virus in cattle has met with little success. This report deals with the development of a procedure for the titration of FMDV in swine.

MATERIALS AND METHODS

The swine in all experiments weighed between 30-70 pounds and were obtained from a herd of line bred Tamworth stock. Difficulties in restraint were experienced if animals weighing over 80 pounds were used, especially during the inoculation of the feet.

Infected bovine tongue epithelium served as the source of virus material. Two subtypes of type O and one of type A FMDV were studied. Subtype M 11, a type O virus recovered from a Mexican outbreak, was used in the comparison of the sensitivity to infection of different inoculation routes. The other strain of type O and the strain of type A, designated as O-39 and A-119, respectively, were received from England as stock cattle strains. Suspensions were prepared by grinding the epithelium with sterile alundum in tryptose phosphate broth at pH 7.4. The suspensions were clarified by centrifugation at 3,000× g for 20 minutes, and the titration series were prepared by decimal dilutions in broth.

In the comparison of the sensitivity to infection with virus by different routes of administration, one milliliter was inoculated intramuscularly in the rump (IM), subcutaneously (S.Q.) in the area behind the ear, or intravenously (IV) in an ear vein. Intranasal (IN) exposure was accomplished by using a syringe without a needle and instilling 0.5 ml in each nostril.

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well inside the nares. In these comparisons eight swine, housed together in the same pen, were used for each virus dilution providing two animals per route of inoculation. After inoculation, observations were made twice daily for the appearance of lesion on feet, mouth, or snout and the final examination of all animals in a room group completed six hours after appearance of lesions on any animal in the room. This provided reasonable assurance that the reactions observed were due to inoculated virus. The route comparison experiments were replicated three times and the results pooled providing six observations per dilution for each route studied.

The feet were inoculated by introducing a 22-gauge, one and one-half inch needle through the coronary band (CB), deep to the hoof wall about one-quarter of the way back from the toe to the heel and directed toward the toe (Figure I). Following this technique and using one foot (two sites) for

![Inoculation of Foot-and-Mouth Disease Virus in the Pig's Foot for Titration of Infectivity.](image)

each dilution, a total of eight observations for endpoint determinations were made per animal. Swine used in foot titrations were housed together and examined 24 hours after inoculation for positive sites. A positive reaction consisted of pronounced swelling and vesication of the coronary band adjacent to the inoculation site. The 50 percent infective virus dilution endpoint was calculated by the method of Reed and Muench (2).

Simultaneous with the swine inoculations the virus was titrated intradermolingually in cattle and intraperitoneally in mice following the methods of Henderson and Skinner, respectively (1, 3).
RESULTS

Results of experiments comparing the sensitivity of the different routes of inoculating are summarized in Table I. The data note that swine were less sensitive to infection with the FMDV strains used than were cattle, or mice but that virus was best detected in swine by inoculation of the feet. The reactions of the intramuscular, intravenous, and subcutaneous routes to the detection of virus were about equal, but the low sensitivity to infection by the intranasal route is noted.

Table I shows the distribution of positive sites of a factorial arrangement of inoculations to determine if different feet, animals, or groups of animals were inoculated.

### TABLE I

**Comparative Sensitivity of Different Routes of Inoculation in Swine to Foot-and-Mouth Disease Virus**

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Replicate</th>
<th>Mean</th>
<th>Comparative Resistance Cattle = 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>CB</td>
<td>5.9*</td>
<td>5.7</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>4.0</td>
<td>3.0</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>4.2</td>
<td>4.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>SQ</td>
<td>4.0</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>Cattle</td>
<td>IDL</td>
<td>8.7</td>
<td>8.6</td>
<td>9.4</td>
</tr>
<tr>
<td>Mice</td>
<td>IP</td>
<td>8.0</td>
<td>7.9</td>
<td>8.9</td>
</tr>
</tbody>
</table>

* Reciprocal of Log10. Fifty percent infective dose endpoint per ml of inoculum.

Route of inoculation: CB = coronary band, foot; IM = intramuscular; IV = intravenous; SQ = subcutaneous; IN = intranasal; IDL = intradermal lingual; IP = intraperitoneal.

### TABLE II

**Factorial Arrangement of Virus Dilutions to Determine Comparative Sensitivity of Animals, Groups and Feet of Swine Using the Foot Inoculation Titration Technique**

<table>
<thead>
<tr>
<th>Group I</th>
<th>Animal No.</th>
<th>Left Front</th>
<th>Right Front</th>
<th>Left Hind</th>
<th>Right Hind</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>281</td>
<td>*5/ +/ +</td>
<td>4/ +/ +</td>
<td>7/ +</td>
<td>6/ +</td>
</tr>
<tr>
<td></td>
<td>278</td>
<td>7/ + +</td>
<td>5/ +/ +</td>
<td>6/ +</td>
<td>4/ +/ +</td>
</tr>
<tr>
<td></td>
<td>277</td>
<td>6/ + +</td>
<td>7/ + +</td>
<td>4/ +</td>
<td>5/ +</td>
</tr>
<tr>
<td></td>
<td>282</td>
<td>4/ + +</td>
<td>6/ + +</td>
<td>5/ +</td>
<td>7/ +</td>
</tr>
<tr>
<td></td>
<td>276</td>
<td>7/ + +</td>
<td>4/ +/ +</td>
<td>6/ +</td>
<td>5/ +</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>5/ + +</td>
<td>7/ + +</td>
<td>4/ +</td>
<td>6/ +</td>
</tr>
<tr>
<td></td>
<td>275</td>
<td>6/ + +</td>
<td>5/ + +</td>
<td>7/ +</td>
<td>4/ +/ +</td>
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<tr>
<td></td>
<td>279</td>
<td>4/ + +</td>
<td>6/ + +</td>
<td>5/ +/ +</td>
<td>7/ +</td>
</tr>
</tbody>
</table>

* Indicates 50 percent infective endpoint reciprocal Log10 per ml virus dilution inoculated.

† One observation for each half foot.
FOOT-AND-MOUTH DISEASE VIRUS IN SWINE

varied significantly in sensitivity to infection. Swine were allocated to two groups of four animals each. Inoculation sites were chosen by random selection except that no inoculated dilution would appear in any pig or any one foot location of each group more than once. Endpoint calculations based on the results of these experiments are noted in Table III. Statistical calcu-

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Number of Animals</th>
<th>Sites Bracketing Endpoint</th>
<th>Fifty Percent Endpoint Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>8</td>
<td>32</td>
<td>5.4*</td>
</tr>
<tr>
<td>Group I</td>
<td>4</td>
<td>16</td>
<td>5.4</td>
</tr>
<tr>
<td>Group II</td>
<td>4</td>
<td>16</td>
<td>5.1</td>
</tr>
<tr>
<td>Left fore foot</td>
<td>8</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>Right fore foot</td>
<td>8</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>Left hind foot</td>
<td>8</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>Right hind foot</td>
<td>8</td>
<td>8</td>
<td>5.2</td>
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<td>4</td>
<td>6.5</td>
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<tr>
<td>Animal No. 275</td>
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<td>5.5</td>
</tr>
<tr>
<td>Animal No. 279</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Reciprocal Log10.

lations based on these data show that a four-fold difference between two endpoints was significant at the five percent level if four animals per titration were used.

The analysis of variance based on nine endpoint determinations of two different strains of type O and one strain of type A is shown in Table IV. A highly significant difference (p < .01) in endpoints was indicated among the strains of virus studied. Separation of means into significant and non-significantly different groups indicated that type O subtype M 11 FMDV titered 17 to 30 times higher in swine than O-39 or A-119.

**DISCUSSION**

The utilization of the swine foot for the titration of FMDV is dependent in part on the sensitivity of the area. The comparison of the titration of virus inoculated by different routes indicates the validity of the assumption that the swine foot is highly sensitive to infection with foot-and-mouth disease virus. Consideration must also be given to the number of observations a technique provides for the determination of an endpoint. It is undesirable for economic reasons to use one animal for each dilution. The use of the
Analysis of Variance of Swine Foot Titration Endpoints of Three Different Strains of Foot-and-Mouth Disease Virus

<table>
<thead>
<tr>
<th>Virus Type and Strain</th>
<th>0</th>
<th>0</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6.7*</td>
<td>5.1</td>
<td>4.3</td>
</tr>
<tr>
<td>II</td>
<td>5.9</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>III</td>
<td>6.2</td>
<td>4.9</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses</td>
<td>2</td>
<td>3.8423</td>
<td>1.9200</td>
<td>11†</td>
</tr>
<tr>
<td>Trials</td>
<td>2</td>
<td>.0023</td>
<td>.0011</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>.6974</td>
<td>.1743</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>4.5420</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as reciprocal Log_{10}. Fifty percent infective endpoint dilution.
† Significant at one percent level.

Swine foot, providing eight observations per animal, makes the proposed titration technique feasible for the assay of FMDV infectivity with results reproducible within good statistical limits (S.D. ± .34 log units) when four animals are used per titration. The use of more animals is not justified in most cases as 16 animals would be required to reduce the error by half.

The described titration procedure will have particular application in the assay of virus concerned in FMD vaccine studies, and tests for innocuity and potency of vaccines. It is generally noted that current foot-and-mouth disease vaccines prepared for use in cattle are of little value in swine due to poor immunizing qualities. The data presented show that the bovine-passaged viruses studied were about 1,000 times more infective for cattle than for swine. Titrations using the described technique have shown that after the single passage of bovine virus in swine the infectivity for swine can be increased tenfold. This indicates that the problem of FMD vaccination in swine may be primarily concerned with the origin of virus used in the vaccine rather than in differences due to immune response of animal species.

**SUMMARY**

A technique for the titration of foot-and-mouth disease virus in swine by inoculation of the feet is described. Validity for the procedure was based on a comparison of different routes of inoculation which showed that virus could be detected in higher dilution by foot inoculation than by intramuscular, intranasal, subcutaneous, or intravenous routes. Application of the procedure in comparison of viruses of different types and subtypes...
indicated that quantitative infectivity differences existed. The application of the technique to FMD vaccine studies in swine is noted.

REFERENCES


INCIDENCE OF VESICULAR DISEASES IN UNITED STATES

In 1952 one day of the National Assembly of this organization was spent in discussing the problems relative to the control and eradication of vesicular exanthema. We are proud to report that probably the only comments during this entire meeting will be contained in five paragraphs of this report since this disease was declared to be eradicated on October 22, 1959. The last known case of vesicular exanthema in the United States occurred in November 1956. Without the cooperation between the various state governments and the federal government, plus the support of the swine industry, the eradication of this disease would not have been possible. All who have contributed to this outstanding accomplishment are to be congratulated.

Just one word of warning—it should be recalled that the VE virus previously has demonstrated capability of surviving in some way for long periods of time. Consequently, we strongly recommend continued vigilance in the inspection of garbage-fed swine in this country.

Due to reduction in funds, personnel on the VE program in the various states have been reduced. We urge that the inspection of garbage feeding premises be assigned to personnel currently on state and federal rolls so that they will continue to be completely covered.

Unfortunately, the records show that as time goes on more and more states continue to show a reduction in the number of inspections made of their premises on which garbage is fed to swine. We should not lose sight of the fact that even though VE has been declared eradicated, enforcement of laws and regulations governing cooking of garbage should be unrelenting in order to lessen the transmission of other important diseases, such as hog cholera and trichinosis.

Nor should we lose sight of the fact that an established program of garbage cooking is a vital precautionary measure in preventing introduction and dissemination of diseases, such as foot-and-mouth disease, African swine fever, and others, that can be spread through the feeding of garbage. According to available evidence foot-and-mouth disease was introduced into the United States in 1914, 1924, and 1929 through the feeding of garbage containing infected meat.

VESICULAR STOMATITIS

There was an extensive outbreak of VE in southeastern Texas during late spring and early summer of 1959. Epizootiological studies of the condition
showed the disease to exist in adjacent areas in Mexico. The outbreak was quite severe and the morbidity in cattle and horses was exceptionally high. The interest and cooperation of the Mexican regulatory officials were outstanding and they are to be commended for the part they played in the identification and control of the outbreak within their country.

Sporadic cases were reported in the New Orleans stockyards, as well as in the endemic areas in Georgia and South Carolina.

VESICULAR DISEASE RESEARCH DEVELOPMENTS

Formaldehyde, combined with high relative humidity and high temperature, was shown to be an economical and efficient method of fumigation where surface decontamination (1) is of primary importance.

It was found that procomplementary swine serums when phenolized and stored became anticomplementary when used in the complement-fixation (CF) test. A 1:2500 dilution of merthiolate preserved the serums satisfactorily and did not interfere with the CF test.

VESICULAR EXANTHEMA

With the eradication of VE, research on it will be discontinued at the Beltsville laboratory. A paper is being presented at this meeting on some of their latest work. All VE viruses at the Beltsville station have been transferred to Plum Island.

Some recent work by Dr. R. A. Bankowski has shown that after the eleventh passage of a strain of virus identified as 101 (1943 isolate) which was stored as infected epithelium in glycerin phosphate buffer at plus 4°C for 10 years and nine months, then prepared as a five percent suspension and inoculated intradermally into the snout, was still infectious for all three of the inoculated pigs.

We now know that there are at least 11 types of VE viruses and two more that have not as yet been checked against all of the known types to determine if they are different.

RESEARCH DEVELOPMENTS

VESICULAR STOMATITIS

Comparative studies on the CF and serum-neutralization (SN) activity and persistence of titer in serums obtained from horses and cows following infection with vesicular stomatitis led to the following conclusions:

1. Measurable CF and SN antibody titers appeared six to eight days postinoculation and reached their peak in nine to 15 days, and
2. Horses and cows were positive to the CF test for not more than 110 days following infection, whereas the same animals were SN positive for several years.

Dr. Lars Kartsad described a human infection that occurred during the Texas outbreak. In combination with the information we have obtained from
Georgia it appears that VS of man is relatively common as a naturally-occurring disease in areas in which livestock become infected. Attempts to isolate VS virus from insects captured on farms in Texas where the disease appeared this year failed. However, the collection of insects was not at the time that the disease was in its most active stage.

Work is being done to investigate the agar-gel precipitin test for VS. Work continues on studying the feral pig as a primary reservoir of VS in the endemic areas within the United States.

**RESEARCH DEVELOPMENTS**

**FOOT-AND-MOUTH DISEASE**

Critical studies at Plum Island (2) have confirmed the resistance to heat of at least one strain of FMD virus. By injecting comparatively large quantities of heated epithelial suspension by various routes, it was found that heating as high as 85° C for as long as six hours was required to totally inactivate the virus. The minimal quantity of infective virus could only be demonstrated, however, when masses of the heated virus were injected into several cattle.

Published information on chemical inactivation of FMD virus has been reviewed at Plum Island (3). Studies in this field are being prosecuted vigorously with the hope of developing improved procedures. (Until proved differently we will continue to use one to two percent lye.)

Significant studies of purification of FMD virus have been accruing. Two distinct particles have been demonstrated in the virus system by several workers at different laboratories, using such methods as electron microscopy ultracentrifugation, agar-gel precipitin tests and chromatography (4, 5, 6, 7, 8, 9). It has been rather conclusively shown by several techniques that of the two known particles in the system, one approximately eight mu in diameter is essentially non-infective and fixes complement in the presence of type specific antiserum. The larger particle, approximately 22 mu in diameter, is infective and it also fixes complement.

Several workers have reported isolation of infectious ribonucleic acid (RNA) from FMD-infected tissues and tissue culture (10, 11, 12). The infectious RNA has been stripped of its protein coat with phenol, and studies are progressing toward determination of specific properties of the RNA and protein fractions. Preliminary evidence indicates that the RNA of the virus is resistant to acid conditions (10, 12). It has also been shown that intact tissue culture virus may be inactivated at 100° C for five minutes, but infective RNA still may be obtained from the boiled virus (12).

Studies at the Plum Island Animal Disease Laboratory have been conducted to determine persistence of FMD virus in boned, salt-cured meat, processed in accordance with commonly-used commercial procedures (13). These studies have shown that the virus may persist in residual blood and lymph nodes in such cured meats for at least as long as 50 days, and in bone marrow of refrigerated carcasses for considerably longer periods. On account of these results, importation in the U. S. A. of such cured meats, from countries where FMD exists, has been prohibited.
VESICULAR DISEASES

Workers at the Pirbright laboratory in England have compared the effects of formaldehyde and acetyl ethylenimine on antigenicity and infectivity of FMD virus. In gel preparations, it was shown that antigenic structure was relatively unaltered by acetyl ethylenimine, whereas with formaldehyde it was altered markedly. An experimental lot of vaccine in which the imine was incorporated for inactivation was superior to a vaccine treated with formalin (14), when tested in guinea pigs.

At the Federal Instituto for Research on Virus Diseases, in West Germany, an ingenious and promising method is being explored for evaluation of FMD vaccines. Adult mice are inoculated intraperitoneally with varying quantities of the vaccine. After three weeks the mice are inoculated with virus of the same type as in the vaccine. Adult mice do not succumb to FMD, but if not protected do develop a viremia, which may be measured by subinoculation of blood from the adult mice into suckling mice. Determinations of the degree of protection against viremia form the basis of the assay (15). Further investigations may permit practical evaluations of FMD vaccines in mice rather than as it usually has been done in cattle.

Studies recently reported from the Central Veterinary Institute, Amsterdam, Holland (16), have shown that FMD virus may be recovered from the saliva of a substantial portion of recovered cattle for at least as long as five months following infection. In some instances infective virus was recovered even though the animals had been vaccinated in the meantime.

A series of studies on infectivity of the viruses of FMD and VS for domesticated birds have been summarized recently (17). In these investigations it was shown that chickens, turkeys, guinea fowl, ducks, and geese may be infected experimentally with either of these viruses, and that lesions may develop on the tongue and foot pads. Spread of infection among these birds was not observed, nor were clinical infections seen in chickens or geese placed in close contact with cattle infected with FMD. Experiments on chickens with VE virus were negative.

Comparatively few controlled studies have been made of FMD in swine. Often, however, these animals have played an important part in past epizootics of the disease. Accordingly, basic studies of the infection in pigs are under way at Plum Island. A system of titration of the virus in swine and a comparison of methods of exposure are being reported at this meeting.

REFERENCES


PROPOSED AMENDMENTS TO THE CONSTITUTION AND BY-LAWS

J. G. MILLIGAN

Montgomery, Alabama

AMEND ARTICLE V—OFFICERS

38 The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Secretary-Treasurer, and an Executive Committee.

77 The President-Elect shall be ex-officio chairman of the Executive Committee.

AMEND ARTICLE VII

103 2. President-Elect: The president-elect shall be chairman of the Executive Committee. In the absence of the president, he shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the president he shall perform all duties of the president. He shall be an ex-officio member of the Executive and Program Committees.

109 3. First Vice-President: The first vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president and president-elect. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect. He shall be an ex-officio member of the Executive Committee.

115 4. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, president-elect and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of the president-elect, and first vice-president. He shall be an ex-officio member of the Executive Committee.

DR. G. H. GOOD: Your Committee on Nominations has met and we are pleased to nominate the following members: for President, Dr. James R. Hay, Chicago, Illinois. For First Vice-President, Dr. A. P. Schneider, Boise, Idaho. For Second Vice-President, Dr. W. L. Bendix, Richmond, Virginia. For Third Vice-President, Dr. T. J. Grennan, Jr., Providence, Rhode Island. The Nominations have been approved by all Members of the Nominating Committee.

MR. BUZZELL: Are there any nominations from the floor.

DR. A. K. KUTTLER: Mr. President, I move the acceptance of the report of the Nominating Committee.

DR. R. W. SMITH: I second the Motion.

PRESIDENT BUZZELL: It has been moved and seconded that we accept the nominations as given by the Nominating Committee. Is that your pleasure? If so, you may manifest it by saying "aye". Those opposed, the same sign. The motion carries. Members of the convention, we now have a new President—Dr. James R. Hay; a new First Vice-President, Dr. A. P. Schneider; a new Second Vice-President, W. L. Bendix; and a third Vice-President in Dr. Tom Grennan. Now the Chairman of the Nominating Committee will make nominations in industry representation for the Executive Committee.

DR. G. H. Good.

DOCTOR GOOD: The Amendment to the Constitution and By-Laws pertaining to delegates at large to be elected to represent the livestock industry, including poultry, as members of the Executive Committee of the United States Livestock Sanitary Association was approved by the majority of the voting members present at the meeting of this Association held in San Francisco, Thursday, December 17, 1959. It provides that only two delegates each from each of four districts of the United States are to be nominated. Your Committee on Nominations hereby submits the following names of candidates for your consideration: Southern District, Mr. John Armstrong, Elkhart, Alabama, a beef cattle producer; Mr. James Nance, Los Alamos, Tennessee, swine producer. The North Central District, Mr. Merriman Steddom, Grainger, Iowa, a swine producer; Mr. Arney Agnew, Milton Junction, Wisconsin, a dairymen. Western District, Mr. J. S. Brenner, Grant, Montana, beef cattle producer; Mr. O. H. Timm, Dixon, California, sheep producer. Northeast District, Mr. Fred J. Nutter, dairymen; and Mr. Alfred Hubbard, a poultryman from Walpole, New Hampshire. The above list of nominees has been approved by the Membership present from the various districts represented on the Nominating Committee.

DR. T. C. GREEN of West Virginia: Mr. Chairman, I move the acceptance of this report.
DR. J. E. STUART, California: I second the motion. President F. G. Buzzell, is there any discussion; are there any other nominees. You will be prepared to bring in your ballots for these people as nominees.

DR. R. W. SMITH of New Hampshire: Mr. Chairman, I move that the Secretary be instructed to cast one ballot for the entire slate and that that shall constitute the vote of this Convention.

DR. H. G. GEYER of Ohio: I second the motion.

PRESIDENT BUZZELL: Are you ready for the question. You may manifest your approval by saying "aye"; those opposed same sign. Motion carried.

DR. RALPH HENDERSHOTT: As instructed by you, I herewith cast the vote of this Assembly for the men listed, as Nominees by the Nominating Committee and as Members representing the farm industry on the Executive Committee as follows: Dr. James R. Hay, President; Dr. A. P. Schneider, First Vice-President; Dr. William L. Bendix, Second Vice-President; Dr. Thomas J. Grennan, Jr., as Third Vice-President. As Members on the Executive Committee from the Southern District, Messrs. John Armstrong and James Nance; from our Central District, Messrs. Merriman Steddom of Iowa and Arney Agnew of Wisconsin; from the Western District, Messrs. J. S. Brenner of Montana and O. H. Timm of California; from Northeast District; Messrs. Fred Nutter, dairyman, and Alfred Hubbard, poultryman, of Walpole, New Hampshire.

PRESIDENT BUZZELL, of Maine: At this time I would like to have the officers elected come up to the rostrum; also any members of industry present who were elected as industry representatives. While these men are making their way to the platform I wish again to express my thanks to all of the people who have taken part in the program and assisted on the Committees for this year. I have had wonderful support. I hope your support will be continued for the officers elected today, and if you have anything to say, you now have the floor.

DR. J. R. HAY: Thank you, Mr. President. Ladies and Gentlemen, I think it is customary at this time to first of all call upon the new officers of this organization to see whether they have anything to say. Dr. Tom Grennan, as Third Vice-President, do you care to make a few comments.

DR. W. L. BENDIX: Thank you, Mr. President. I heartily concur in that this Association is worthy of the best efforts of all of us. I certainly and sincerely pledge mine. Thank you very much.

DOCTOR HAY: The next man is the hardest working member of the whole group, being the Chairman of the Executive Committee. Dr. A. P. Schneider, would you care to say a few words?
REPORT OF

DR. A. P. SCHNEIDER: President Hay, I want to take this opportunity to thank all of you and assure you that I will give my utmost to perform the duties that you have given. Thank you very much.

DOCTOR HAY: I believe that it is customary for a new President to express himself by saying that he accepts the responsibility and that in all humility that he will work in the best interests of the Association for the ensuing year and certainly I do not wish to change the pattern. This is all true for 1960. However, there is one modification which I wish to make that is a little different from the usual procedure. At this time I would like to announce the Chairmen of the Committees for the coming year. Now, these Chairmen will have the prerogative of selecting the members of the Association whom they wish to serve with them during 1960. It is imperative that they have in the hands of the Secretary not later than January 15th the names of those people they may wish to have serve with them. Now, a number of them have already given their list to Doctor Hendershott and those of you who have not, I urge you to accomplish this by the fifteenth of the next month. The Committee Chairmen are as follows: Anaplasmosis, Dr. M. N. Riemenschneider, of Oklahoma; Committee on Biologics and Pharmaceuticals, Dr. N. H. Casselberry, of Berkeley, California; Committee on Brucellosis, Dr. R. W. Smith, State Veterinarian, Concord, New Hampshire; Committee on Infectious Diseases of Cattle, Dr. H. W. Johnson, United States Department of Agriculture, Beltsville, Maryland; Committee on the Nationwide Eradication of Hog Cholera, Dr. C. L. Campbell, Box 389, 439 Monroe St., Tallahassee, Florida; Committee on Laws and Regulations, Dr. J. W. Safford, Helena, Montana; Committee on Leptospirosis, Dr. E. Roth, Department of Veterinary Science, L.S.U., Baton Rouge, Louisiana; Committee on Nominations, Dr. A. L. Brueckner, College Park, Maryland; Committee on Transmissible Diseases of Poultry, Chairman, Dr. H. E. Goldstein, Ohio Department of Agriculture Laboratory, Reynoldsburg, Ohio; Committee on Parasitic Diseases, Dr. V. D. Chadwick, State Veterinarian, Jackson, Mississippi, Chairman; Committee on Public Health, Dr. R. J. Schroeder, 9744 Atlantic Ave., South Gate, California; Committee on Public Information, Dr. R. L. Knudson, Apartment 10, 1505 W. 28th St., Arlington 6, Virginia; Committee on Rabies, Dr. E. S. Tierkel, United States Public Health Service, Atlanta, Georgia; Transmissible Diseases of Sheep and Goats, Dr. E. A. Tunnicliif, Bozeman, Montana, Chairman; Committee on Stockyards, Markets and Transportation, Dr. J. J. Martin, Chairman, United States Department of Agriculture, Washington 25, D. C.; Transmissible Diseases of Swine, Dr. L. A. Rosner, Chairman, Jefferson City, Missouri, State Veterinarian; Committee on Tuberculosis, Chairman, Dr. R. W. Carter of Box 1174, Columbia, South Carolina; Committee on Livestock Integration, Chairman, Dr. A. L. Sundberg, State Veterinarian, State House, Des Moines, Iowa; Committee on Vesicular Diseases, Dr. F. J. Mulhern, United States Department of Agriculture, A.D.E., Agricultural Research Service, Washington 25, D. C.

This completes the Committee Chairmen at this time. In accordance with the wishes of the Executive Committee, there may be additional committees appointed. However, we are not prepared at this time to announce the chairmen. I certainly appreciate the help that has been bestowed upon me and I will turn the meeting back to Mr. Buzzell. Thank you.
2nd ANNUAL MEETING
CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS
DECEMBER 14-15, 1959
SAN FRANCISCO, CALIFORNIA

WILLIAM L. SIPPEL, President, Kissimmee, Florida
E. P. POPE, Secretary, Madison, Wisconsin

1. Rabies Diagnosis, JAMES L. McQUEEN.
2. Laboratory Confirmation of Bovine Tuberculosis, G. N. LUKAS.
3. Differential Diagnosis of Swine Diseases, PAUL BENNETT.
4. Epidemiology and the Diagnostic Laboratory, HARRY E. GOLDSTEIN.
5. Tissue Culture Methods as a Diagnostic Tool, R. A. BANKOWSKI, et al.
6. Toxicological Techniques for the Diagnostic Laboratory, A. A. CASE.
7. The Diagnosis of Listeriosis, JOHN W. OSEBOLD.
8. Laboratory Diagnosis of Anthrax, H. B. ELLIOTT.
9. Leptospirosis Diagnosis—Present and Future, ERSKINE V. MORSE.
10. Laboratory Notes on the Diagnosis of Anaerobic Bacterial Diseases, EDWIN M. ELLIS, et al.
RABIES DIAGNOSIS
SPECIAL APPLICATION OF FLUORESCENT ANTIBODY TECHNIQUES

JAMES L. McQUEEN, D.V.M.*

INTRODUCTION

It was shown in the 1958 and 1959 publications of Goldwasser and Kissling et al. (1, 2) that fluorescent antibody techniques can be used to identify street and fixed rabies viral antigens in the brain tissue of experimentally infected mice and in the salivary tissues of naturally infected animals. On the basis of this work, the Florida State Board of Health has undertaken to apply fluorescent antibody techniques to the routine laboratory diagnosis of rabies. This report amplifies the preliminary report on this study, which was presented at the American Public Health Association meeting during October of 1959 (3).

THE FLORIDA STUDY

The primary objective of this study was to evaluate the Fluorescent Rabies Antibody (FRA) test † as a practical, routine diagnostic procedure under conditions expected to exist in the majority of state public health and other diagnostic laboratories.

As a practical consideration, we were concerned only with the identification of rabies virus and/or viral antigens in the brain tissues of naturally infected animals. Sellers' stained smears, prepared from all fresh or frozen brain specimens submitted to the central laboratory for rabies diagnosis, were examined microscopically for Negri bodies. The FRA test was run independently to determine the presence of rabies viral antigens. These results were compared with the results of standard mouse inoculation tests, which were, for the purposes of this study, considered as the definitive criteria of the presence or absence of infection (3).

Source of Reagents:

The principal reagent, known as conjugate, was produced according to previously described procedures (2, 4, 5) by labeling hyperimmune anti-rabies globulin solution (Lederle) ‡ with Fluorescein Isothiocyanate (6), (Baltimore Biological Laboratories and Sylvana Chemical Company).‡

* Senior Assistant Veterinarian, Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Atlanta, Georgia, on assignment to the Florida State Board of Health.

† Hereafter referred to in the text as the FRA test.

‡ Reference to Companies or to trade names of products does not constitute endorsement and is used for the purpose of identification only.
**Microscopic Equipment:**

A Reichert "Fluorex" unit,‡ employing an Osram HBO 200 mercury vapor lamp and fitted with a Corning 5840 (half-stock thickness) filter, served as the light source. The standard monocular microscope was used with a cardioid dark-field condenser. The objectives, complete with funnel stops when needed, were a 10X dry, a 45X dry, and a 97X oil immersion. The 10X ocular was fitted with a Wratten 2-B gelatin filter (3).

**Processing of Specimens:**

In preparing slides for FRA examination, two very thin impression smears were made from Ammon's horns and/or cerebral cortex on each of four slides. The smears were air dried for 30 minutes and were then fixed for four hours in reagent grade acetone at minus 20 degrees C.; after which time the slides were removed from the acetone and allowed to dry thoroughly while remaining in the freezer (2).

**Staining of Smears:**

The direct staining procedure of Coons and Kaplan (5), as modified by Goldwasser *et al.* (1, 2) was used exclusively in this work. Stated briefly, two slides of each specimen were removed from the freezer. The right or test smear on each slide was covered ml to 0.04 ml of a mixture of equal parts of conjugate and normal mouse-brain tissue suspension (NMB), and the left or control smear on this slide was treated similarly with a mixture of equal parts of conjugate and rabies-infected mouse-brain tissue suspension (IMB). The slides were incubated for 30 minutes at 37°C., and then washed 10 minutes in two changes of phosphate buffered saline (pH 7.2-7.4) to remove excess tagged antibody. The slides were then air dried and a drop of glycerol-saline mounting fluid and a cover slip were added (3).

**Control Systems:**

The system of controls used in the Florida study was divided into three distinct levels or phases. Phase one was concerned primarily with determining the sensitivity and proving the specificity of a finished conjugate. At the second level of control, a check was made to reaffirm conjugate sensitivity and specificity on the day of the test. The third level of control was exercised as each slide on the individual unknown specimen was stained and examined.

With respect to the first control phase, as each lot of conjugate was produced and before it was used in the test system, it was tested against known positive (rabies infected) and negative specimens. Only conjugates which produced specific, distinct bright staining of rabies viral antigen in known positive specimens and from which the non-specific staining properties had been almost totally eliminated were regarded as satisfactory for use. Further proof of conjugate specificity was obtained by demonstrating: (1) that the conjugate would not stain normal brain tissue or brain tissue in which inclusions of disease other than rabies were demonstrable, (2) the inhibition
or partial inhibition of staining by pretreatment of a known positive smear with unconjugated anti-rabies serum; and finally, (3) the complete inhibition of staining by the addition of rabies-infected mouse-brain tissue suspension to the conjugate (5, 2, 3). It should be noted here, that the exposure of conjugate (labeled antibody) to rabies-infected tissue suspension (homologous antigen) acted as the basic control mechanism for the entire test system. Tissue suspensions were prepared of normal and rabies-infected mouse brain according to those procedures described by Goldwasser, et al. (2). Prior to application of the conjugate to the smear, aliquots of the conjugate were mixed with equal parts of the normal mouse-brain tissue suspension (NMB) and with rabies-infected mouse-brain tissue suspension (IMB). The mixing of conjugate with IMB suspension completely inhibited the staining (attachment of antibody to virus antigen) of known positive specimens, whereas the similar use of NMB suspension produced no such inhibitory effect (5, 2, 1, 3).

Each group of unknown specimens was accompanied by a known positive and negative slide. As a re-check on conjugate specificity at the second level of control, the known negative was read first. To reaffirm the day-of-test sensitivity of the system, the known positive was then examined. With the system working correctly, no greenish-fluorescent particles were noted in either the test or control smears of the negative specimen. In contrast, varying numbers of bright green fluorescent bodies ranging in size from the barely visible to those over 20 microns in diameter, were readily apparent in the known positive test smear (treated with conjugate—NMB). The positive control smear (treated with conjugate—IMB) exhibited only the grey-blue to greenish grey autofluorescent background of unstained or normal tissue (3).

When reading the unknowns, the test smear was first examined and in the absence of greenish particulate fluorescence the control smear was not consulted. When greenish fluorescent particles were observed in an unknown test smear, they were examined more closely on higher magnification (X970) to determine more precisely their morphological characteristics. The control smear of this specimen was then read and identical staining reactions occurring on both the test and control smears were regarded as non-specific. Here then, we introduced the third phase or individual-slide level of control. That is, if the unknown did in fact contain rabies virus, we should note characteristic greenish fluorescent particles in the test smear, whereas the control smear would show no fluorescence if the reaction was specific. It is apparent also that greenish fluorescent particles should be absent from both the test and control smears in a negative specimen (3).

Reading of Unknown Specimens—Observations:

The aggregates or accumulations of viral antigen as observed in unknown field specimens assumed various shapes and sizes. As reported by Goldwasser and Kissling (1), the size and shape of the larger fluorescent particles were closely correlated with the size and shape of the Negri bodies seen on the direct microscopic examination of Sellers' stained companion smears. It
should be emphasized, however, that uncountable numbers of smaller fluorescent particles, which were unidentifiable by Sellers' staining, were observed in every positive specimen. In a few cases, particularly if the specimen was Negri negative, the positive FRA diagnosis was rendered on the defined specificity of these smaller antigen aggregates alone (3).

The phenomenon most frequently observed on the examination of rabies-positive specimens was the presence of round or nearly round bodies, exhibiting bright peripheral fluorescence with central darkening or mottling. These morphologically distinct "ring forms" were observed in every positive specimen with such a high degree of regularity that they were virtually diagnostic (3).

Results and Analysis of Data:

The diagnostic efficiency of a rabies test may be calculated by determining its sensitivity and specificity. That is, a test detecting all and only diseased (rabies virus containing) specimens is considered to be 100 percent efficient. For purposes of the study, the mouse inoculation test was considered to be the definitive criterion for the detection of rabies virus and was used as the standard of comparison.

A total of 884 fresh or frozen brain specimens have been processed at this writing. Of these specimens, 82 have been confirmed rabies positive by mouse inoculation (Table I).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mouse Inoculation Negative</th>
<th>Mouse Inoculation Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>221</td>
<td>42</td>
</tr>
<tr>
<td>Fox</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Cat</td>
<td>215</td>
<td>3</td>
</tr>
<tr>
<td>Raccoon</td>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>Skunk</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Bat</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>Bovine</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bobcat</td>
<td>1</td>
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<td>Horse</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Human</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Other*</td>
<td>199</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>802</td>
<td>82</td>
</tr>
</tbody>
</table>

* Squirrel 72, Opossum 27, Goat 2, Monkey 4, Hamster 13, Rat 61, Gopher 1, Mouse 3, Rabbit 13, Mole 2, Mink 1.
As a means of evaluating the efficiency of the FRA test, the results of the FRA test were compared with the results of the mouse inoculation test (Table II). It may be seen from Table II that the FRA test detected all of the 82 positives and included in its positive results only specimens which were confirmed positive by mouse inoculation. Therefore, the FRA test is equally as sensitive and specific (efficient) for the detection of rabies virus and/or viral antigen in the brain tissues of naturally infected animals as is the mouse inoculation test.

Comparisons were also made between the results of Negri body examinations and mouse inoculations (Table III). It may be seen from Table III that Negri bodies were demonstrable in only 76 of 82 specimens confirmed rabies-positive by mouse inoculation. Thus, the sensitivity of the Negri body test, when compared to the mouse inoculation test, was calculated \( \frac{76}{82} \times 100 \) at 92.7 percent. It should also be noted from Table III that virus isolates were obtained from only 76 of the 86 specimens recorded as Negri positive. Therefore, the specificity of the Negri body test, when compared to the mouse inoculation test, was calculated \( \frac{76}{86} \times 100 \) at 88.4 percent.

Combining the figures for sensitivity and specificity \( \left( \frac{76}{82} \times \frac{76}{86} \times 100 \right) \), we determined the Negri body test to be 90.5 percent efficient for the detection of rabies virus in the brain tissues of naturally infected animals.
RABIES DIAGNOSIS

DISCUSSION

In the past, it has been necessary to produce the primary reagents in the individual laboratory. The preparation of fluorescent tagged antisera (conjugate), although not without pitfalls, is neither particularly difficult nor impractical. It is hoped however, that in the near future, the demands for quality controlled rabies conjugates will be met by commercial outlets. At this writing, at least one company (Sylvana Chemical Co.) has rabies conjugate listed among their fluorescent antibody reagents. It should be pointed out that at present, commercial conjugates are shipped in an unadsorbed state. It will therefore be necessary, unless otherwise directed, for the individual laboratory to perform the tissue powder adsorptions according to accepted procedures (4, 2) and to test varying dilutions of the finished conjugate against known positive and negative specimens to obtain an optimal working dilution.

It should be stressed that the choice of optical equipment is of the utmost importance to successful performance of the test. The unit should utilize the HBO 200 mercury vapor lamp or a lamp comparable in intensity and wave length potential. For rabies work, and virus work in general, a simple monocular microscope fitted with a cardiod darkfield condenser appears to give excellent results. The very small amount of visible light emitted by a rabies smear may be lost to the prism system of a Binocular microscope unless the instrument is highly refined. Since the color and intensity of the background fluorescence (tissue auto-fluorescence) may be greatly altered by the characteristics of the exciting light, a filter system for rabies work must be chosen with great care. The field or primary filter should transmit light in the near ultra-violet range, i.e., between 3500-4000 angstroms. Primary filters permitting the transmission of violet-blue light (4100-4700 angstroms) are to be avoided. The ocular or secondary filter should be nearly colorless yet opaque to ultra-violet light (3).

Although the exact nature of non-specific staining reactions is poorly understood, these reactions may be broadly classified as (1) staining of normal tissue cells, (2) diffuse staining of the tissue; and, (3) precipitation of stain. The undesirable staining of normal tissue cells must be almost totally eliminated by tissue powder adsorption if the conjugate is to be considered satisfactory for use. The staining reactions caused by the second and third factors are usually morphologically distinct and easily differentiated by the experienced person from a specific reaction.

Although much information may be gleaned from published material (1, 2, 3) on techniques and procedures and application of the FRA test, it will be advisable for each laboratory to build upon their own experience with these. If possible, persons working with the FRA test should gain first hand experience in the preparation of reagents. Using a conjugate of proven specificity and sensitivity, inexperienced workers will need to stain and read a great many known positive and negative specimens before they may become proficient and considered qualified to read unknowns. It would seem advisable, at least until the efficiency limits of the FRA test have been precisely
defined, to correlate the results of all Negri body examinations and FRA tests with the results of standard mouse inoculation tests.

SUMMARY

It has been shown previously that fluorescent antibody techniques can be used experimentally to successfully demonstrate the presence of rabies virus in brain and salivary tissues (1, 2). The work presented here describes the Fluorescent Rabies Antibody (FRA) Test as applied to the detection of rabies viral antigen in the brain tissues of naturally infected animals. The usefulness of the FRA test as a rapid, practical, routine diagnostic procedure is emphasized.

The FRA test was in complete agreement with the results of the mouse inoculation tests conducted on the total of 884 specimens representing 23 different species of animals. The FRA test detected rabies viral antigens in all of the 82 positives, whereas Negri bodies were demonstrable in only 76 of 82 or 92.7 percent. No false positive reactions by FRA were recorded, whereas 10 specimens from which no virus could be isolated were recorded as Negri positive.

Results would indicate the FRA test to be entirely comparable in efficiency to that of the mouse inoculation test for the detection of rabies virus in the brain tissues of naturally infected animals. In contrast, the Negri body test was only 90.5 percent efficient for the demonstration of specific rabies virus or viral antigen when compared with the results of standard mouse inoculation tests.

It is emphasized, by comparing the test specificities of the FRA and Negri body tests, that the FRA test may be used advantageously to differentiate between a true rabies inclusion (Negri body) and inclusions of other diseases which are sometimes quite difficult or impossible to define by Sellers’ staining.

The exacting standards of performance so necessary to successful application of the FRA test, are directly related to the training, experience and proficiency of the diagnostician as well as to the quality of the reagents and equipment employed. Exercising sufficient care the experienced person can, with carefully controlled reagents and the proper optical equipment, use the FRA test to render a highly specific rabies diagnosis in a day’s time. The advantages of this test, which combines the speed of histological techniques with the sensitivity and specificity of biological assay, are obvious to all engaged in the field of preventive or corrective medicine.

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LABORATORY CONFIRMATION OF BOVINE TUBERCULOSIS

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As veterinary laboratory diagnosticians our primary responsibility is to establish whether the suspect material is tubercular; therefore, we are obligated to use the more or less proven procedures. This report does not describe any spectacular or startling new methods to confirm bovine tuberculosis. The field force, concerned with bovine tuberculosis is not interested in probabilities and suppositions but whether the tissue obtained from a reactor is tubercular. Suspect material is reported at once as positive if it shows typical macroscopic tubercular lesions and morphologically typical acid-fast bacilli are demonstrated by direct smears. Often we culture and section such specimens but further laboratory confirmation is purely of academic interest.

The specimen that does not demonstrate acid-fast bacilli by direct smear is processed by digestion and concentration with subsequent bacteriological culture and animal inoculation to determine the presence of viable tubercle bacilli. Histopathology is done if there is sufficient material to work with.

Upon reviewing the available literature and the results from our limited laboratory work, I believe that the initial digestion, neutralization and concentration of the specimen is the most critical part of processing suspect material. Unfortunately, the veterinary laboratory diagnosticians, in contrast to the workers in human medicine, are dealing with tissue that is bulky, fibrous and often grossly contaminated during examination and removal from the carcass. Therefore, digestion must not only accomplish maceration and dissolution of the adjacent glandular, fatty, elastic and fibrous tissue of the tubercle, but decontamination without destroying the mycobacterium. Our work is further complicated by the fact the density of the organism is only slightly greater than that of water. It has been reported that the tubercle bacilli are found in the supernatant in solutions of density greater than 1.4 when centrifuged for 30 minutes at the speed of 8,000 rpm, but are spun down when the density of the liquid is 1.2 to 1.3. This finding resulted in Darzin applying calcium phosphate precipitation with simple centrifugation to recover the tubercle bacilli.

With this information in mind, we have included the described "shaking precipitation technique" along with the classic sodium hydroxide digestion and centrifugation for concentration of the micro-organism. Enzyme digestion has been tried with limited application. Recently, Merck and Company provided this worker with a proteolytic enzyme called "Ficin" and we are attempting trials on the efficacy of this material for the digestion of tubercular specimens.
Gianforte and co-workers recently described the use of X-108, a powerful proteinase, as an aid in primary isolation of bacteria. The results reported warrant investigation of the use of this latter enzyme in tubercular tissue digestion.

Because each worker has a medium or combination of media he prefers, this report will not discuss the relative merits of the various culture methods. We have used commercially-prepared Lowenstein-Jensen medium and have prepared other described formulae. In addition to the various egg media, many liquid media have been tried with variable results. Slide cultures, using Kirchner's and also Darzin's liquid media have been relatively successful but obviously the slide culture cannot tell us the probable role and pathogenicity in the bovine. Other than using this latter technique to demonstrate an acid-fast organism we do not routinely include this method in our workup of suspect material. Recently, Stonebrink's egg media described by Lesslie (1) has been prepared and is currently included in the culture setup in an attempt to evaluate its usefulness as compared to the standard media.

With the obvious limitations of the direct smear and the somewhat erratic results from bacteriological cultures, and the failure of the histopathological sections to demonstrate viability and pathogenicity of the bacilli, the test animal inoculation trials assume their proper perspective in that they serve as the culture medium as well as demonstrating viability and pathogenicity.

Doctor Traum and many other investigators have repeatedly emphasized the significant role the test animal plays in confirming tuberculosis. It has been pointed out that extremely low numbers of organisms in the inoculum will cause an infection in the animal whereas comparable inoculums will fail to demonstrate these bacilli by culture and/or direct smears. Therefore, we depend on the results of the animal inoculation to determine the presence or absence of viable organisms in the suspect material.

It is interesting to note that many workers who have proposed methods and specific formulae for culture media have used the results of the animal inoculation trials to demonstrate the efficacy of their methods and media. Therefore, one can conclude that the animal inoculation will demonstrate the presence of a tubercle whereas culture or histopathology may fail. This fact is one of the reasons why the state laboratories have relied on the use of the guinea pig in confirming bovine tuberculosis. In the course of the routine workup of the specimen in addition to confirming tuberculosis, we make an attempt to establish the cause of the granulomatous lesions such as coccidiomycotic, actinomycotic or lesions resulting from probable parasitic invasion. Many tuberculin reactor cattle from a more or less given area in California showed lesions simulating tubercles which were confined primarily to the mesenteric lymph nodes. The relative consistency of the appearance of these lesions and the unusual nature of the problem prompted considerable effort to prove or disprove the presence of tubercle bacilli. Countless direct smears, histopathological sections, cultures and animal inoculations did not demonstrate a pathogenic acid-fast bacillus. The macroscopic lesions (slides 1-7 inclusive) resemble tubercles enough so that every person who has initially observed them has diagnosed them to be lesions resulting from
tuberculosis, but upon closer scrutiny of these lesions, one will observe the peculiar greenish to dark brown discoloration and the somewhat defined encapsulation. The microscopic pathology (slides 8-13 inclusive) shows varying degrees of granulocytic predominately eosinophilic infiltration in a more or less circumscribed area and ultimate encapsulation. In presenting the microscopic sections, we have attempted to show the progressive development of these eosinophilic granulomas.

The central valley area can be considered as having coccidiomycosis as an endemic problem. This is reflected in the numbers of suspect tubercular lesions that are primary coccidiomycotic granulomas. We demonstrate this organism by wet mount and histopathology. The mycotic media are always contained in screw top culture tubes and are not opened for observation because of the potential danger to the technicians.

The actinomyces are demonstrated in suspect material and occasionally it and the former organism are found in the same lesion.

All of us are aware that the visible tuberculin reaction is not always indicative of the extent of infection evidenced by gross lesions in the animal. Along this line, our laboratory recently received specimens from a Johne's reactor and non-reactor. The specimens were correctly identified evidenced by the collector's gross description of his findings. The tissue from the reactor showed a thickening and conjection of the mucosa and the tissue from the non-reactor showed a slight inflammation adjacent to the ileocecal valve (slides 14-19 inclusive). Direct smears from comparable sections from each specimen showed relatively few organisms in the reactor whereas each field of the direct smears in the non-reactor showed many thousands morphologically typical of mycobacterium paratuberculosis. The microscopic sections confirmed the observation of the direct smears.

In confirming bovine tuberculosis and, as illustrated in the above Johne's findings, the gross appearance, microscopic findings are simply aids and in the end then (slide) it is the guinea pig who is the master performer in this chaotic procedure we call diagnosis.

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Differential diagnosis of swine diseases could be made the subject of a rather lengthy discussion depending upon the number of diseases to be covered and the detail with which each disease might be described. I am sure that this particular group does not need any such lengthy dissertation even if I were inclined and capable of going to such lengths. Instead, please let me confine my remarks to only two swine diseases which I have selected for different reasons. One of them is hog cholera which has been known for many years and has proven to be an extremely important and costly disease to the swine industry. It is still the number one swine disease problem in spite of efforts throughout all of these years to bring it under satisfactory control. As a diagnostic problem it is still the most difficult swine disease known. The other disease I have selected is Mycoplasma infection. This is a comparatively recently recognized swine infection, some of the characteristics of which, I believe, mark it as a potential trouble maker for the swine industry. At the present time the importance of the condition might be described as in the incubative stage.

Ten years ago severe losses due to hog cholera stimulated increased activity in the study of the disease. During that 10 years there has been almost a complete change in the biological products used for immunological purposes. This present year of 1959, however, is an appropriate time to point out that the availability of new products has not changed the attitude of owners in their long established practice of decreased vaccination activities during a period of less favorable market prices. Many reports this year have pointed out that there was a large increase in the number of unvaccinated swine. Neither have the changes and the studies of the past 10 years added anything of practical value to our diagnostic capabilities. They have added evidence to previously known features of the disease which we had only infrequent occasion to remember. They have made us more conscious of the natural variations that occur. They have improved our ability to recognize and evaluate those variations, but all this added evidence needs to be emphasized and re-emphasized to gain more widespread diagnostic value.

Such things as chronic cases of cholera; instances of high percentage of recovery; infective strains of low virulence; strains of very high virulence; and variations in symptomatology have all been observed and reported for many years. Ten or more years ago all of these things apparently did not occur with sufficient frequency that they were considered important enough to be given equally important emphasis with the so called typical or average cholera outbreak. Naturally, they did not get equal attention with the average case in educational information about
the disease and present day recognition of cholera is partly a matter of unlearning the textbook picture. Present day cases of cholera are often of the chronic or low virulence types. In fact, in our laboratory a majority of the cholera cases fall into one or another of these categories. It is not at all uncommon for the present day case of cholera to be very unlike the well known typical or textbook description of the disease as far as symptoms and gross lesions are concerned. The low virulence cases are especially liable to be wrongly diagnosed because of the presence of some secondary or concurrent disease.

These cases can be so entirely atypical in the matter of symptoms and gross pathology that no one can identify all such cases without the benefit of animal inoculation tests. One new feature of animal inoculation test methods has been found necessary during the investigations of the past few years. We formerly believed that cholera could be diagnosed by the injection of two test pigs, one of which was immunized and the other was susceptible. If cholera virus was present in the injected material the immunized pig would remain normal and the susceptible pig would become sick and develop recognizable evidence of cholera. Now it is often necessary to retain the susceptible pig and give it a challenge dose of virulent virus, because the low virulence strains do not cause recognizable symptoms but can produce a solid immunity. From questions which have been asked, some people apparently suspect that the increase in number of cases of low virulence might be connected in some way with the commercial development of vaccines of low virulence. Since this low virulence type of infection began appearing in numbers more than two years before the modified viruses became available it does not seem possible that the new products had anything to do with the current cases. If changes in the characteristics of disease producing agents can be caused by man, it is quite probable that the same, as well as other changes, can occur more prolifically in nature.

Anyone who is strongly influenced in his diagnostic methods by the average picture of cholera may find it difficult to recognize the occurrence of the disease in pigs as young as one week old. Such cases should be readily suspected when the parent stock is unvaccinated, however they also occur in vaccinated stock. The degree of immunity following vaccination, and its duration, is subject to considerable variation. A few instances have been observed in which immunity has disappeared in the parent stock, either on an individual or a drove basis, and cholera infection occurs. Recognizable symptoms and loss may or may not occur in the parent stock, but losses usually do occur in their young off-spring, and it is quite easy to miss the basic reason for these baby pig losses. These cases usually raise questions regarding the possibility of a variant strain being involved. The answer to this question depends on just what is meant by the term variant. None of the cases observed in Iowa during the past two or three years have been characterized by the events that occurred 10 years ago when this terminology became popular. There are undoubtedly different antigenic patterns just as there are different pathogenicity patterns, but personally I am not yet
convinced that antigenic differences go beyond the limits of these pattern variations.

Since many current cases of cholera can be considered as atypical when compared with the well known average case of several years ago, where can we put our finger on something of diagnostic value while remodeling our picture of an average case. The first three things to be considered in diagnostic procedures include history, symptoms, and lesions. Of these, the history has undergone the least change from the average picture and I think it is here that we need to pay particular attention and study to secure the best and most recognizable evidence of cholera infection. From the diagnosticians viewpoint two features of the over-all cholera situation stand out as highly significant factors at this time. First, many cases of cholera are unrecognized, and second, we still need a dependable, accurate, and relatively simple procedure for diagnostic purposes.

Mycoplamosis, or as it is more familiarly known, PPLO infection, of swine is a comparatively recent addition to the list of swine diseases. Some may recognize it under the name Glasser's disease but it should be emphasized that Glasser's disease and Mycoplasmosis are very probably two separate diseases with grossly similar lesions. Glasser's disease has been recognized in Europe since 1910 and is apparently caused by Hemophilus suis. Switzer (1953) has shown that the mycoplasmosis to which I refer is evidently caused by Mycoplasma species.

In contrast with cholera, this disease is easily diagnosed by gross lesions and cultural methods. Clinically however, the symptomatology resembles that of other diseases. It has become common in Iowa swine and if it follows a similar pattern of development in other swine producing areas, should be increasing in those areas.

The incidence and loss in any individual drove is usually erratic, intermittent, or sporadic. The disease may affect swine of any age and losses can occur in pigs as young as three weeks old.

The infection appears to have a high degree of preference for serous membranes and gross lesions are commonly found on the heart, pleura, peritoneum, and meninges. Another location in which the infection is found rather often is the joints of the legs. Observable symptoms usually can be traced to one or another of these lesion locations. Joint infection soon causes symptoms of arthritis. The affected joint becomes slightly swollen and contains an excess of fluid. This fluid is clear but somewhat thicker, gummier, and more cohesive, than normal. There is usually no erosion of the articular surfaces. Joint infection is practically sure to result in observable symptoms but they can be easily mistaken for arthritis due to some other organism or to injury. Infection involving the meninges also results in observable symptoms easily associated with the central nervous system. Again, excess fluid is present and a whitish opacity and thickening of the membranes may be detectable.

When the heart, pleura, and peritoneum are affected the symptomatology is much less distinct and in several cases may not be noticeable at all. Individuals of very good general appearance may be found dead without any
apparent reason. During periods of excitement or more than the usual amount of exercise an individual pig may fall over dead.

In other cases individual animals may rapidly fail to continue their previous thriftiness and well doing state of development. Except for this unthriftiness they may continue to appear and act normally for a few weeks before a worsening of their condition becomes evident. Other individuals may develop respiratory difficulties and in still others hydrothorax or ascites develops.

In early stages of the infection the exudate formed may be a soft, cheesy, but cohesive accumulation. More commonly the lesions observed are fibrinous and very adhesive. This adhesiveness is especially prominent in the thoracic cavity where the pericardium and pleura become practically integral parts of organs and other tissues with which they come in contact. In some instances it is difficult to believe that the heart or lungs could have any freedom of movement.

In relative frequency of occurrence of lesions in the various anatomical locations, the pericardial and pleural lesions rank first. Next in frequency is the arthritic infection. All cases have not been examined for meningeal lesions so our information on this is incomplete and not reliable. The peritoneal lesions can be found quite frequently but their apparent severity and damaging effect seems to be rather minor when compared with the extent and damage resulting from the infection in other locations.

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EPIDEMIOLOGY AND THE DIAGNOSTIC LABORATORY

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The title of this paper in all probability should read, Epizootiology and the Diagnostic Laboratory. However with the terms epidemiology, epizootiology and recently the coinage of epornitiology, we in Ohio have placed veterinary epidemiology in common usage. Therefore, in most instances when we refer to epidemiology in this paper we are using a term more easily pronounced and one that has far more appreciation in discussions with allied groups.

Epidemiology may be said to have reached its modern development when the laboratory became an integral part of field investigation of disease.

The elementary function of epidemiology is to determine the frequency and distribution of mass disease, thus providing the needed information for organization, guidance, and evaluation of animal health programs. The same principles of veterinary epidemiology, may and are applied to an individual herd or flock disease problem, just as to a number of herds or flocks located on many different premises.

In Ohio, the Division of Animal Industry has a section devoting its full responsibility to veterinary epidemiology. This section has three phases of operations. The pathological services laboratory; the animal disease morbidity-mortality reporting program; and the field investigative services, are integral parts in the over-all epidemiology section.

The morbidity-mortality reporting program affords the information as to what diseases are occurring, where the diseases are occurring, so far as geographic distribution, and how much disease is occurring, regarding incidence rates.

The pathological laboratory can substantiate the accuracy of the reporting program by confirmative diagnosis, as well as by stimulating the reports from initial diagnosis.

When the needs arise for consultative investigative service or when additional epidemiological information is needed the investigative service becomes active in correlating the laboratory with the field problem.

The practice of veterinary epidemiology can both be spectacular as well as routine endeavor. However in both instances the same basic principles are applied.

In 1952, Ohio experienced one of the more spectacular type disease problems. The problem of anthrax was presented in our swine population. Much has previously been written regarding this epizootic. Basic principles were applied in the approach to the problem. The diagnostic laboratory provided this by affording a bacteriological diagnosis of anthrax within 48 hours of the initial field investigation. In a very few days anthrax had
been diagnosed by laboratory confirmation on 22 premises in one county and in less than three weeks had been diagnosed in 55 counties on 422 premises.

In this problem the diagnostic laboratory performed a lion’s share of the responsibility in evaluating the problem. The next phase was to uncover the source of a disease which had not been endemic to the state.

By careful use of epidemiological survey it was determined that a common denominator on each premise existed. This common denominator was a specific feed ration. The laboratory was again called upon to perform bacteriological cultures as well as feeding trials on the components of the ration. To make a long story short, by laboratory efforts the contaminated feed material was found to be raw meat scrap.

In many epidemiological problems the results are not of the spectacular nature. Prominent features of a developing epidemiological problem for chronic type diseases place great importance on ecological principles in determining disease behavior, increased recognition of both stress and management changes, and more need of statistical evaluation of these observations.

A disease problem that can be placed in this category is that of “jowl abscess” in swine. This problem is very costly to the meat packer and ultimately effects the producer in the market price he receives.

In Ohio, we have been very concerned with this problem. It has not been difficult at all to culture the lesions and afford bacteriological evaluations. Streptococci, staphylococci, and corynebacterium have routinely been cultured from the jowl abscess lesion. Preventive measures as well as adequate treatments have been the real problem to hurdle. In our early epidemiological approach to the problem we incriminated the self-feeder as a predisposing stress for this condition, but when confronted with the same problem where the self-feeder was not used we modified our thinking.

Truly, the jowl abscess problem is dependent upon a predisposing stress. In using the epidemiological approach to this predisposing stress we are in the process of gathering data on all phases of management practices where jowl abscess occurs. This includes surveying breeding records with the approach that the stress may be of a genetic nature. All feeding, watering, bedding, and pasturing practices are being given a careful scrutiny. All management practices are being correlated to observe the possibility of a common denominator stress.

This is not the spectacular type of laboratory epidemiology, as much of the data must be given careful evaluation when correlated by attack rate procedures. It is routine and will take several years before any of the data may take on any significance. In each case laboratory studies must be conducted to ascertain that all cases are the same condition.

In many of the problems which are presented to our laboratory we are observing that stress factors are beginning to closely over-shadow the disease classics. These stresses are most difficult for laboratory personnel to evaluate. We have a philosophy that in many instances negative laboratory reports are just as significant as positive findings. In eliminating the disease, by the
same procedures as affording the positive diagnosis, we have in turn focused our thinking to environmental stress.

A field approach to many of the problems is that of determining pre-disposing stress for a given condition. However the laboratory must be an active participant in evaluating the field problem.

It must be pointed out that even though the laboratory findings are an important adjunct to any epidemiology problem, the epidemiologist must evaluate and correlate the laboratory findings with the field problem.

We had an interesting problem presented that involved the death of four beef cattle. One animal was presented for necropsy. The gross necropsy findings revealed lesions in the area of the scapula marked by an adematous, gaseous, type lesion. Bacteriological cultures of this area afforded cultures of *Clostridium Chauvoei*. Blackleg was incriminated from the laboratory findings, however, the history of the case indicated differently. The farm was in a blackleg area and the disease had occurred on this farm in past years. The field investigator observed that a herbicide had been used along the fence rows to control weed growth. The nature of the kill of weed growth indicated that an arsenic preparation had been used. Samples of the fence posts and large weeds were taken to the laboratory where extensive amounts of arsenic was determined by chemical analysis. The liver and kidney of the second animal also was positive for arsenic. The investigator was then able to obtain positive information from the neighbor that a herbicide containing 26 percent sodium arsenite had been used on the fence rows.

This example points out the need for the epidemiologist to have an open mind in evaluating the problem. The laboratory findings in epidemiology are a most important part in defining the problem, but must be checked and re-checked in order not to confuse the field picture, but rather to more clearly define the problem.

In many epidemiological problems the laboratory must assume the role of duplicating field conditions in setting up field studies. This probably could be labeled as research but actually is more of a field study type project.

In the most northern area of our swine belt in Ohio we have made a study of Transmissible—Gastro—Enteritis in baby pigs. This has been a most costly problem to our swine industry. The high incidence rate occur at 5 to 6 year cycles with lesser numbers of farms involved each year between the high years.

The onset of the disease has been observed on many farms after the first hard snowfall. The snowfall has been correlated with the feeding habits of the starling. When the snowfall covers the small grain and weed fields, the starling moves into the feedlot with the hogs. On 66 farms in one county we were able to correlate this phenomena with the onset of transmissible gastro enteritis.

Therefore, we set out to attempt to reproduce the disease in susceptible baby pigs from feet washings and the intestinal contents of starlings which were shot and trapped on farms where the disease occurred.

The results were inconclusive as all the control pigs developed the disease and rendered our results unworthy of reporting. A more extensive study
will be conducted this year. The study is only mentioned as depicting the role the laboratory must assume in evaluating epidemiology problems.

Along these lines of duplicating field conditions a very interesting problem was presented to our laboratory involving high mortality in pheasants, due to nitrate poisoning.

During the early spring of 1957 a majority of the rearing fields at the Waterloo Wildlife Experiment Station Athens County, Ohio, were plowed, fitted and planted on April 28 to various cover crops. These pheasant rearing fields were heavily fertilized at the time of planting with 12-12-12 granulated fertilizing at the rate of 400 pounds per acre. After the fertilizing and planting the rainfall for Athens County was comparatively low during the following 10 weeks.

Rainfall for Athens County from April to July was 7.60 inches as compared to 14.42 inches of normal rainfall based on a 40 year average for this same period. Due to this dry condition the granulated fertilizer kept its original consistency.

The pheasant brooding and rearing schedules were designed in such a manner that at 45 days of age the chicks were transferred from brooder houses and sun porches to conditioning pens. Prior to this age only two percent of the young chicks had been lost due to normal mortality. The first transfer to conditioning pens was on July 16 and consisted of 1,000 ringneck chicks.

The week prior to transfer the temperature reached 112° F. at ground level for two consecutive days. Twenty percent mortality occurred during these two days in other species of pheasants, mainly Reeves' and Elliot's (Syrmaticus reevesi and S. ellioti) but ringneck mortality was normal.

On July 19, three days after the ringneck chicks were transferred to conditioning pens the birds began showing signs of nauseaion. Feed consumption dropped to less than one-half while water consumption more than tripled. This was noticed at 2:00 P.M. and at 2:30 chicks began dying at the rate of about 30 birds per hour. This mortality continued until dusk.

Dead and live birds were rushed to the Reynoldsburg Diagnostic Service Laboratories for autopsies. Post-mortem observations showed slight hemorrhages in the muscles of the breast and thigh. These are typical of mild hemorrhagic disease (stress diseases). Laboratory studies showed that bacteriological cultures of the heart, liver, and lung tissues were negative for any significant growth. Chicks were put on a water medication of Terramycin as an aid in checking the slight hemorrhages. The following day July 30 the remainder of the original 1,000 chicks died before evening.

Investigators from the laboratory went to the experimental station the same day, and autopsied 120 pheasants. Not one of the chicks showed signs of disease except for a few scattered hemorrhages in the breast and thigh muscles.

Temperatures that day rose to 102° F. It was suspected that the 1,000 birds lost had undergone injury to their thermo-regulatory system the previous week when the temperature reached 112° F. It was theorized that,
if this were true, the chicks would be unable to adapt themselves to extreme
temperature changes. Death could be a result of this injury.

On July 26, 1,000 additional 45 day old chicks were transferred to another
conditioning pen. Four days later the birds became prostrate and comatose
showing symptoms to that of the birds 10 days previous. By noon on
August 1, 61 dead birds were picked up from the pens. Again, autopsy
showed only a few slight hemorrhages in the breast and thigh muscles.
Klotojen W. and anticoagulant, was administered in the drinking water this
time as an agent to check hemorrhage.

The following day only seven birds died. On August 3, 200 birds died in
the morning as the temperature rose to 103°F.

During the day, four experiments were set up to try different medications
(sulfa drugs, NF 180, antibiotics) and sparing tranquilizers. Half of the birds
used in the experiments were showing symptoms of the comatose-prostration
syndrome, while half of the birds were still apparently normal, and were
feeding. The affected birds were hand-fed during the evening. The following
morning all birds appeared to be perfectly normal and were back on feed
and water. There was no difference in the various sample groups regardless
of the medication or tranquilizer used.

Additional non-treated birds were sent to, Reynoldsburg Laboratory,
Battelle Memorial Institute, and Ohio State University for autopsy. All reports
showed that birds were in apparent good physical condition except for a
few scattered hemorrhages and occasionally a few oocysts of coccidia.
Laboratory studies showed bacteriological cultures of the brain, heart, liver
and lung tissues showed negative for any significant growth.

On the following day 88 chicks died in the forenoon. A water spray
system was set up during the morning and 7,000 gallons of water was sprayed
over the pen. This was an attempt to hold the temperature down. Mortality
had ceased by 2:00 P.M. and birds were eating again. The following day
the pen was sprayed again but birds continued dying. Three days later,
regardless of the type of medication, spraying, and use of tranquilizers, all
of the original 1,000 had died except the 100 birds used in the medication
and tranquilizer experiment. Not one of these birds had died. The experi-
mental birds were being held in isolated brooder houses.

Additional birds of younger age were taken from brooder houses and
put in with the experimental birds to explore the possibility of a communicable
disease. All birds remained in excellent physical condition and no mortality
occurred.

Ten days later 1,000 more 45 day old ringneck chicks were transferred
from brooding units to the flight pen where there had been no birds since
the pen had been seeded early in the spring. Prior to this transfer the
complete diet was changed including the source of drinking water. The
previous diet had consisted of a commercial feed, chick starter 28 percent
protein with two pounds of NF 180 per ton and .0175 percent of sulfam-
quinoline. This starter was fed with scratch grain as 10 percent of the diet.
This diet was changed to Farm Bureau Chick developer—16 percent protein
with no medications of scratch grain added. Included in this transfer were
300 Reeves' pheasants. The third day after transfer the ringnecks were
showing the same symptoms as did the other birds. The pheasants would first lose use of their legs then their wings and neck would drop. After this they would remain in one position. It was suspected that starvation or thirst was the immediate cause of death after the bird reached a state of immobility. A few minutes prior to death the birds developed a respiratory condition which resulted in a few sneezes and coughs.

The remaining birds of this lot were put on the same medication and Sparine tranquilizer regimen as the birds that had survived from the preceding group. This time there was no effect and birds continued to die in large numbers. Again, 100 of these pheasants were transferred to brooder houses. These birds were left on a straight diet of 28 percent chick developer without medication and mortality ceased.

This more or less confirmed the theory that the birds were getting something toxic after they left the brooder houses. The vegetation within the pens was tested by the Laboratory but no known toxic substances were found.

The following week 1,200 ringnecks were transferred to winter holding pen which had also been reseeded and fertilized in the spring. Here again, on the third day after transfer, the birds began dying.

By this time it was apparent that the birds weren't dying until after they had eaten away some of the cover and exposed to the soil. After exposure the birds would begin to dust and pick in the soil. It was now certain that they were picking up undissolved granules of fertilizer from the soil.

Immediately some of the soil was placed in the brooder houses where the experimental birds were being held. In three days the experimental birds began dying. 12-12-12 fertilizer was also sprinkled over the feed in other buildings and three days later birds developed the same symptoms.

The 300 Reeves' pheasants in the same flight pen where 1,000 ringnecks had died still maintained 100 percent survival and showed positively no symptoms. After long periods of observation it was apparent that the Reeves' did less picking at the soil and spent nearly all their time walking the fence while the ringnecks were dusting and picking.

Two hundred ringneck pheasants were obtained from the Urbana Game Farm and subjected to a pen at Waterloo where the soil had been exposed. Within 24 hours 60 percent of these birds had died.

Samples of the soil were taken and analyzed at the Reynoldsburg Laboratory. Tests showed that nitrates were present in amounts that could be assumed to be lethal.

It was suggested that a complete laboratory experiment be conducted with the pheasants in which they would be subjected to nitrates. Birds were sent to Reynoldsburg where the same tests were run using fertilizers mixed in their feed. The same symptoms resulted at the laboratory.

In this problem a great amount of epidemiological effort was expended in correlating mortality rates with climatic conditions as initially all workers were of the opinion that heat prostration was the cause of death. Nitrate poisoning was the farthest from anyones thinking and could not have been substantiated but for a correlated field epidemiological approach coupled with laboratory procedures.

Epidemiology is dependent upon the diagnostic laboratory.
TISSUE CULTURE METHODS AS A DIAGNOSTIC TOOL—
WITH PARTICULAR REFERENCE TO NEWCASTLE DISEASE
AND VESICULAR EXANTHEMA VIRUSES *

R. A. BANKOWSKI, H. IZAWA and J. HYDE

During the past decade tissue culture methods have made great strides as a means of cultivating viruses \textit{in vitro}. The growth phenomenon is not only a method for studying the agents per se but was amenable to procedures which could be applied to diagnosis. This new field owes much to the pioneers in the early part of this century who were interested in tissue culture techniques to study the morphology of cells. In 1928 the culture of viruses in living tissue was made practical by Maitland, who demonstrated that vaccinia virus could be propagated in tissue fragments suspended in a mixture of serum and a balanced salt solution (1). Since then other viruses were applied and improvements of the tissue culture technique were incorporated. Contaminants were controlled with antibiotics, which solved the problem of maintaining bacterial sterility, and the modification and simplification of the media and equipment provided a method of studying viruses which could be performed in most laboratories.

In spite of the great improvements in the media and the cells for culturing specific viruses in a tissue culture system, it is impossible at the present time to describe a single procedure that can be universally or even widely employed by all workers in the field of virology. Tissue and cellular tropism, our lack of knowledge of the influence of media components upon viruses, antiviral substances in sera or extracts of animal origin that are used in the culture, etc., limit this discussion to specific techniques that are currently used in our laboratory, which are also or may be adapted to other agents as diagnostic or research tools. Those who are interested in using tissue culture methods or their application are referred to any one of a number of reviews for details of the media used and the techniques employed (2, 3, 4, 5, 6).

Briefly, two general types of tissue culture procedure that may be most useful to a diagnostic laboratory are:

(1) The Maitland suspended cell method in which fragments of tissues are suspended in a nutrient fluid consisting of a balanced buffered salt solution and a growth promoting substance such as embryo extract or serum ultrafiltrates (7).

(2) The fixed cell procedure may be used in various ways:

(a) Fragments of tissue are planted on clotted plasma and bathed in a nutrient medium. The cultures can be prepared on coverslips, or in larger quantities in tubes or bottles depending upon the nature of the problem.

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(b) Dulbecco and Vogt (8) introduced a method in which the tissues were dispersed with the aid of dilute trypsin solution into cells or clusters of cells that are placed and grown directly on the glass wall of a tube or container in the absence of a plasma clot. This procedure expedites preparation of large quantities of tissue culture material in tubes or large flasks from a pair of kidneys of a medium-sized animal.

(c) More recently considerable progress has been made in propagating cells \textit{in vitro}. Once a line of cells is established they can be produced in large numbers for inoculation of tubes or prescription bottles to produce sheets of cells.

The Dulbecco and Vogt modification further showed that a precise number of virus particles in suspension could be determined providing the agent was cytopathogenic. This development not only allowed studies on the dynamics of a virus but also was applicable for diagnostic procedures. The method consisted of inoculating a monolayer of tissue cells in a Petri dish and exposing the sheath of cells to serial dilutions of a virus suspension. The cellular layer was covered with a nutrient agar which localized the virus. The agent upon multiplication destroys adjacent cells until the area becomes visible. This may be accomplished within 24 hours or more depending upon the agent employed. Figure 1 illustrates serial dilutions of vesicular exanthema virus which were inoculated onto monolayers of pig kidney cells. From the number of plaques, the virus concentration per ml of suspension can be readily calculated. Convincing evidence by Dulbecco indicates that each plaque is caused by a single infective virus particle (8).
PRIMARY ISOLATION OF AGENTS

Primary isolation of an agent from suspected infected material is dependent upon many factors; nevertheless, certain viruses are known to be more readily propagated in tissue culture than by animal inoculation. Cox showed that equine encephalomyelitis virus was more readily infective for tissue cultures than for laboratory animals (9). Maden, York, and Mc kercher were able to demonstrate that infectious bovine rhinotracheatis virus (IBR) could be readily detected by primary isolation when suspected material was inoculated into embryonic bovine kidney cultures (10). On the other hand, some agents and particularly some strains of an agent may be more readily detected in the intact animal. As the tissue culture techniques improve, this method would satisfy a rapid and economical means for isolating a suspected organism. As an example, methods for detecting and identifying poliomyelitis virus by primary isolation of the agent from suspected material was studied intensely and accomplished since the organism is highly host specific and the most susceptible animal (monkey) was too expensive and difficult to maintain for routine diagnosis.

Sensitivity of tissue cultures for vesicular exanthema virus. To determine the efficiency of recovering VE by tissue culture methods, vesicular coverings from snouts and feet which were collected from field outbreaks of the disease or preserved from experimentally infected hogs were used as suspected material. Most of the samples, which were preserved in glycine-PO₄ buffer at -6°C, were stored for three to four years. Ten percent suspensions prepared in buffered saline were inoculated (1) intradermally into the snout of swine and into pig kidney tissue cultures which were prepared in (2) sealed tubes and in (3) Petri dishes. The latter were covered

<table>
<thead>
<tr>
<th>Number Samples</th>
<th>Vesicular Lesions</th>
<th>Fever</th>
<th>Antibodies</th>
<th>Tubes</th>
<th>Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>23*</td>
<td>23</td>
<td>—</td>
<td>—</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1†</td>
<td>—</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Minimum titer 5.5 x 10³ pfu per ml.
† Primary lesions and fever in 1 of 2 swine inoculated.
with agar and incubated at 37°C in a chamber flushed with air-CO₂ mixture (plaque assay technique of Dulbecco).

The results showed (Table I) that of 29 samples tested 23 suspensions produced a cytopathogenicity in cultures of pig kidney tissues in the tubes and plates as well as primary vesicles in swine. One sample did not infect the tissue cultures nor show clinical evidence of the disease in swine; however, the inoculated animal presented a fever and specific antibodies in the serum 25 days following inoculation. Another sample produced a cytopathogenicity in the tube tissue culture, but no plaque forming units nor lesions in swine were observed. In this instance the virus was serially passed in tubes and later identified by the neutralization test using known VE antisera. In one instance one of two hogs inoculated presented a small vesicular lesion with no evidence of infecting the tissue culture systems employed. It was interesting to note that the titer of the virus which produced lesions in swine and tissue cultures were at least 5.5 x 10³ plaque forming units (pfu) per ml of suspension and at least five types of the virus were used in this comparative study. There is no doubt that strains of the virus, infectivity titers, and the material used for the test play an important role; nevertheless, this data proved that tissue culture is a highly sensitive system which can be used for primary isolation of some types of vesicular exanthema virus. Furthermore, the virus can be readily typed or differentiated by serological or other tissue culture techniques.

TISSUE CULTURES AND CHICKEN EMBRYONATING EGGS

The limitations of the use of large animals for diagnostic purposes is well known. The introduction of the chicken embryonating egg as a host tissue played a prominent role in the advancement of our knowledge of virus diseases. Henle et al. (11) proved that primary isolations of mumps virus from saliva of patients with acute parotitis was achieved in tissue cultures of HeLa cells with an efficiency and speed that could not be matched with embryonating eggs. In some laboratories, particularly those engaged in poultry diseases investigation, embryonating eggs can become costly and burdensome, which may limit an investigation or diagnosis. HeLa cell cultures, which are readily and easily propagated, appear to be highly susceptible to ND virus. The possibility of using the more rapid and simple tissue culture methods for research and primary isolation of the virus from infected chickens is being investigated. Table II Illustrates the comparative results.

TABLE II

<table>
<thead>
<tr>
<th>No. Sample Tested</th>
<th>Tissue Culture</th>
<th>Virus Isolation</th>
<th>Emb, Egg</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>6*</td>
<td>6*</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

* Virus isolated in each host tissue from same sample.
It is apparent from this study that the GB strain of ND virus, which produces a marked cytolytic effect on HeLa cells can be readily isolated by a less costly and considerably more rapid method of detecting infections. The infected tube of cells can be used for identifying the cytolytic agent with specific immune sera by the haemadsorption test. Further studies are being conducted with the various strains of ND virus to evaluate the latitude and efficiency of this procedure.

TITRATION OF VIRUS CULTURES

Quantitative information of an agent is in some instances highly desirable or even necessary in a diagnostic or investigational procedure. With some agents this could be extremely difficult or prohibitive in animals because of the cost and host specificity.

There are several practical methods available for titrating a virus in tissue culture. Serial dilutions are made of the virus suspension and a given quantity of each dilution is inoculated into a tissue culture system and observed for (1) changes in pH due to infection and/or destruction of the cells with a consequent decrease in metabolism (2) cytopathic changes in a contiguous sheet of cells (3) formation of plaques by the virus in a contiguous sheet of cells overlaid with agar. Of these methods the two latter procedures are probably used more frequently and effectively.

Introduction of tissue culture techniques in VE research provided a simpler means for evaluating the relationship of infectivity titer and virulence. The study of sensitivity of TC and living animals to VE virus included a titration of the suspension prepared from infected epithelium before injecting it into the animals and tissue cultures. It was found that the titer varied from $10^1$ to $10^6$; however, the extent of the lesions in swine was not necessarily correlated with the tissue culture titer, particularly with material having a low virus content.

It is also important to know that serial passage of a virus in tissue culture may result in a change in the characteristics of the agent. Serial passage of the virulent California 11914 NDV in a Maitland type TC resulted in a progressive decrease in virulence which resulted in a completely avirulent, stable, and highly immunogenic agent. This strain was adapted as a vaccine (12). Serial passage of some types of VEV in pig kidney cells exhibited high titers when titrated by the plaque assay methods. However, inoculation of the TC suspensions having titers as high as $2.4 \times 10^8$ by the intradermal or intravenous route failed to produce clinical signs of the disease but rendered the animals immune to a challenge dose of homologous virus (13). From these and other experiences, the influence of exposing a virus to tissue culture upon its characteristics must be considered when these methods are used exclusively.

Titration of NDV and VEV in tissue culture. Figure 2 illustrates a titration of the Montana strain of Newcastle disease virus in tubes of HeLa cells. Cytopathic changes were readily noted macroscopically in 48 hours; however, the final readings are made macro or microscopically (100-150 diameters) after five days incubation at 37°C. After this time the cells begin
Fig. 2. Cytopathic changes in sheets of HeLa cells by serial ten fold dilutions of Montana strain of Newcastle disease virus.

to spontaneously degenerate which can be easily mistaken for virus activity.

A comparative study of titration of several strains of NDV in HeLa cell cultures and 10-day-old embryos is shown in Table III. This preliminary

TABLE III

Comparative Titers of Newcastle Disease Virus in Tissue Culture and Chicken Embryonating Eggs

<table>
<thead>
<tr>
<th>ND Virus</th>
<th>Titer—LD&lt;sub&gt;50&lt;/sub&gt; or CPD&lt;sub&gt;50&lt;/sub&gt;* Per ml.</th>
<th>Trial I—HeLa Cell</th>
<th>Trial I—Chicken Embryo</th>
<th>Trial II—HeLa Cell</th>
<th>Trial II—Chicken Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCND P&lt;sub&gt;11&lt;/sub&gt; †</td>
<td></td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6.3&lt;/sup&gt;</td>
<td>10&lt;sup&gt;5.8&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6.4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Strain Texas 6B HeLa Adapted P&lt;sub&gt;32&lt;/sub&gt; †</td>
<td></td>
<td>10&lt;sup&gt;7.3&lt;/sup&gt;</td>
<td>—</td>
<td>10&lt;sup&gt;5.2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;5.5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Strain Montana Embryo P&lt;sub&gt;6&lt;/sub&gt; †</td>
<td></td>
<td>10&lt;sup&gt;7.3&lt;/sup&gt;</td>
<td>10&lt;sup&gt;9.2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;7.5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;8.6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Cytopathic dose<sub>50</sub>. † Serial passage. ** Tissue culture attenuated ND virus.

trial showed that the titration of ND virus in tissue culture, when read macroscopically after five days, showed in some instances a high degree of correlation when compared with the titration conducted in chicken embryonating eggs. Consideration must be given to the strains of virus employed. A higher correlation was observed with the tissue culture adapted strains (TCND and 32nd passage of strain GB in TC) than with the virulent virus strain (Montana) propagated exclusively in embryonating eggs. The latter gave higher titers in chick embryos than in the HeLa cell cultures. This is
TISSUE CULTURE METHODS AS A DIAGNOSTIC TOOL

not unexpected as the readings were made macroscopically, and it is known that different strains of virus produce a variable gross destructive effect on cultured cells (14). When a strain of virus has been propagated in animals, it may when first introduced into tissue culture exhibit a delayed or incomplete CP effect (14). On the other hand, it is known that some viruses will not produce a cytopathogenicity nor grow in TC. Mott was unable to propagate a strain of VEV in TC, and the virus was irregularly infective for swine (15). The lack of pathogenicity of some strains of a virus may be an expression of the agent and not of the host or present TC system employed.

A comparison of the plaque assay method of Dulbecco and Vogt and the cytolytic effect of the virus in tubes using four types of VE virus showed that the plaque assay technique resulted in titers of 1.5 to 2 logs higher than those obtained by the cytolytic effect in test tubes (Table IV). The results

TABLE IV
Comparative Titers of Vesicular Exanthema Virus by Plaque Assay and Cytopathogenicity on Sheaths of Line and Subcultured Pig Kidney Cells

<table>
<thead>
<tr>
<th>VE Virus (Type)</th>
<th>Line Cell (Madin)*</th>
<th>1st Subculture of Trypsinized Kidney†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plaque Assay Titer</td>
<td>Tube-Cytolytic</td>
</tr>
<tr>
<td></td>
<td>Plaque Assay Titer</td>
<td>Tube-Cytolytic</td>
</tr>
<tr>
<td>E54</td>
<td>2.3 x 10^7</td>
<td>10^6.12</td>
</tr>
<tr>
<td>F55</td>
<td>3.2 x 10^6</td>
<td>10^4</td>
</tr>
<tr>
<td>G55</td>
<td>2.6 x 10^5</td>
<td>&gt;10^5.0</td>
</tr>
<tr>
<td>I55</td>
<td>1.1 x 10^5</td>
<td>10^3</td>
</tr>
</tbody>
</table>

* Skli Pass No. 4 15VI59.
† OS 41 1st Subculture 10V159.

also indicated that the titers found in cell-cultures prepared from a line cell and a first subculture of a cell suspension prepared from trypsinized pig kidneys resulted in identical or highly correlated titers. The plaque assay method of titration has the advantage of displaying the characteristics of the cytopathic effect of the virus (Figure 1). McLain et al. (16) have indicated that the pathogenicity or virulence of the virus was correlated with the size of plaque that it forms. The larger plaques are indicative of higher virulence for swine than the smaller, slower growing clones. This can be further substantiated by the two recent immunogenically distinct types of VE virus, namely H54 and I55, which invariably produced plaques of the smaller variety. Similarly, two types (J56 and K56) of VE virus isolated by Holbrook et al. (17) from extremely mild cases of the disease were characterized by their inability to produce a plaque larger than a few mm in diameter, and the agents were very apathogenic for hogs.

NEUTRALIZATION TEST IN TISSUE CULTURE SYSTEMS

A neutralization test can be applied to a serum-virus mixture provided that the virus can be propagated in tissue culture and is cytopathic. A tissue-culture neutralization test has been of particular interest and importance
in diagnosis and differentiation of vesicular exanthema because, heretofore, infectious material from the field, when available, required inoculation of various species of animals and later subjected to cross-immunity studies in hogs or the complement-fixation test for its identification. No practical method for detecting antibodies in sera of recovered animals was available. It has been recently established that a tissue culture neutralization test can be utilized for detecting antibodies in sera of swine as early as six days following infection with some types of the virus (13). Madin reported that the antibodies reach a peak at 21 to 28 days following inoculation (18).

Whereas the complement-fixation test with swine sera in vesicular exanthema showed a considerable amount of overlapping between the immune types, no cross protection has been shown to exist in the neutralization test between nine immunologically distinct types of vesicular exanthema (19).

Figures 3 and 4 illustrate the neutralization between homologous and heterologous antisera and two new immunologically distinct types of VE virus which were isolated from the same herd of hogs. Type 155 was isolated from hogs fed meat trimmings, bones, and lymphatic tissues from asymptomatic swine slaughtered following an 84-day quarantine period because of a VE outbreak caused by type H54 VE virus (20). The neutralization tests in tissue culture confirmed the immuno-distinctness that was found by cross immunity studies in hogs (13).

When a neutralization test is conducted using a constant amount of virus in the presence of an equal volume of sera diluted arithmetically or in a logarithmic sequence, the relative potency of an antiserum can also be determined (19).

**HEMAADSORPTION TESTS**

The hemaadsorption test first described by Vogel et al. (21) and Shelokov et al. (22) is a rapid and simple procedure which is of considerable practical importance to a diagnostician for the detection of a cytopathogenic virus of the myxo-virus group. The simplicity and amenability of this procedure can be best illustrated with our present technique with Newcastle disease virus. Tubes of HeLa cells are inoculated with the suspected material, prepared and treated with antibiotics as routinely employed for embryonating eggs or animal inoculation studies. After 24 to 48 hours, preferably longer, when the virus attacks the cells and prior to an observance of gross destruction of cells, the fluid medium is removed and the cell sheet washed twice with a buffered saline. To the tube is added 0.2 ml of a 0.4 percent guinea pig red blood cell suspension and the tubes allowed to stand at room temperature for three to five minutes. Examination of the tubes macroscopically or under the microscope using 100 to 150 x magnification presents an aggregation and adsorption of the erythrocytes to the periphery of the infected cells (Figure 5).

* At the time of writing this report, type J56 VEV showed some cross neutralization with types E and C.
FIG. 3.
NEUTRALIZATION OF TYPE H_{54} VEV WITH HOMOLOGOUS AND HETEROLOGOUS ANTI VEV SWINE SERUMS.

FIG. 4.
NEUTRALIZATION OF TYPE I_{55} VEV WITH HOMOLOGOUS AND HETEROLOGOUS ANTI VEV SWINE SERUMS.
Fig. 5. Hemaadsorption of guinea pig erythrocytes to HeLa cells infected with Montana strain ND virus.

The test has been shown to be an effective tool for a number of viruses (influenza, mumps, ND virus, and others of the myxovirus group).

The test is of particular importance to the diagnostician for identification of a virus in a relatively short time with a minimum of equipment and facilities. Viruses having the property of hemaadsorption can also be differentiated and identified with immune antisera. If a number of tubes are inoculated, one or more of the tubes is washed and treated with an antiserum of the suspected disease. After an incubation period of fifteen minutes at room temperature, the red blood cell suspension is added. An inhibition of hemaadsorption can be readily demonstrated with the homologous antiserum-virus combinations. This procedure is rapid and simple, requiring a minimum of manipulation for isolation and identification of an agent. The test procedure can also be readily adapted to conduct a survey on antisera collected from the field. Serum samples can be submitted to the laboratory and used for the hemaadsorption inhibition test in tubes previously inoculated with a known virus such as Newcastle disease and other agents of the myxovirus group.

SUMMARY

It is obvious from this short discussion that the tissue culture technique has already a wide application as a diagnostic tool in veterinary medicine and has enlarged our understanding of the nature and behavior of some
animal disease viruses. Although it is impossible to describe or recommend a single procedure that can be universally used in all laboratories for all agents, modifications of techniques are continually being described which emphasize the fluid situation. The tissue culture technique is not only more economical but is conducive to producing more research in less time, requiring fewer facilities. Nevertheless, the progress that has been made already with the tissue culture techniques supports the belief that eventually a simple, accurate, and economical procedure or procedures will be established which will be more or less universally accepted into the standard methods of diagnostic procedures.

ACKNOWLEDGMENT

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TOXICOLOGICAL TECHNIQUES FOR THE
DIAGNOSTIC LABORATORY

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Toxicology may be defined as the study of poisons in action. It would include both those from a natural source, and those that in one way or other are of economic or industrial use. They may cause their injury in a number of ways, but the ones of most importance from the standpoint of making a positive diagnosis of poisoning are those which are ingested by the animals. It is this group of poisons which are most likely to be a problem for analysis by the diagnostic laboratory. Usually, the toxicological tests are done by the laboratory which is doing the rest of the diagnostic procedures.

There is a world of difference between the small service laboratory which is conducted in connection with a one-man veterinary practice and the large, elaborately equipped diagnostic laboratory which is supported by a regional authority of the federal government or a large commercial organization. Much will depend upon how complete a service the diagnostic laboratory is expected to give. The laboratory able to furnish a "complete" service including every possible kind of analysis, such as trace tests for residues of newer insecticides and other commercial poisons is a rare establishment and its services are costly.

However, such complete services are not often required for the usual analyses performed in the diagnostic laboratory. What is required, as a rule, is an indication to the veterinary clinician that he is on the right approach to the problem under consideration.

There are several syndromes seen in domestic animals that have many symptoms in common so that it is impossible for the veterinarian to be sure just which condition is present, unless he has help from the diagnostic aids of the laboratory (4). It is by taking advantage of the available differential tests, including toxicological analysis, that the course of the condition may be recognized and confusing entities ruled out. Often, the diagnostic laboratory confirms what the clinician already suspects, or helps him to narrow his choice (9).

One of the most difficult problems facing the clinician working with the sick animal is that which includes two or more marginal intoxications, either of which can be easily mistaken for the other. An example of such a complex which is being seen more often in the Missouri area is the problem of excess nitrate in the ration. This can cause trouble in a dairy herd which the clinician may encounter as a refractory milkfever, a loss of production

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simulating mastitis, an A-vitaminosis syndrome which looks like hyperkeratosis (or an outbreak of keratitis), or in animals which die, heavy metal intoxication or leptospirosis. With such a problem, the clinician needs to know definitely that he does not, for instance, have to treat lead, arsenic, or HCN intoxications. What he sees may look like a routine attack of parturient paresis, but the response to the specific therapy for parturient paresis is not effective. Here is where the toxicological techniques really come into their own.

Rather than taking the time to describe in detail the various toxicological tests, emphasis will be placed on "what" to look for, and "how" to go about solving the complex problem as presented by the clinician. The actual technical steps in making any of the tests discussed here have been adequately described in other places (1, 2, 3, 4, 5, 7, 8), so little time will be taken here except to mention which procedures are in order, and how to make an interpretation of the results. Ideal, of course, is the situation where the clinician actually working with the problem in the animals can also perform the laboratory procedures. Usually, this is not possible, so an important requisite for the laboratory tests, especially the toxicology part, is a complete signalment and anamnesis (1, 9). This is especially so, if any forensic procedures are likely to follow.

The proper procedures for taking and forwarding samples for toxicological analysis have been adequately described by numerous authors (Bamford; Kaye; Garner). This is of especial importance if one suspects either accidental contamination of the feed or a malicious poisoning. It is almost as important in instances where commercial poisons have been used in field spraying or in other applications, such as in orchards adjoining pastures. Another instance in which the veterinarian finds himself facing a difficult situation is where animals die following the use of a known nontoxic herbicide, such as 2-4D or its esters. Are such animals dying from ingestion of poisonous plants such as milkweeds, HCN producers such as plum or cherry shrubs, nitrate intoxication because of ingestion of nitrate concentrators, or of some infectious or contagious disease? One will have to be very careful with both his treatment of such animals, and in what he says as comments concerning the condition he thinks is present (8, 9, 10). The veterinarian will most certainly need the help of some toxicological laboratory procedures in such an instance as this.

The other thing that the veterinary clinician has to keep in mind is that, although the diagnosis was the correct one, the source of the trouble may have been entirely different from the one at first suspected (6). This is a common happening, and often proves very embarrassing to the veterinarian, unless he has foreseen that possibility (10).

Good qualitative tests which have been of much value in our area include the following: Reinsch test for heavy metals (10); diphenylamine test for nitrate and diazotization test for nitrite (1, 4, 7); immature or baby crickets for insecticides and allied chlorinated hydrocarbons (11); small laboratory mammals for feeding tests to screen suspected feeds; picric acid-sodium carbonate test for prussic acid (2); the frog test for strychnin (2); and the
cat eye test for black nightshade or Jimson weed poisoning (1, 2). There are many other tests, several of which are useful in special situations (1). While such bioassay tests are not considered to be toxicological techniques as such, they may be very useful to indicate such things as the presence of ergot alkaloids, enterotoxemia toxin or botulinus toxin, vitamin A deficient forage, or other deleterious agents for which specific chemical tests are not readily available (1, 9).

The diagnostic laboratories which are located at such teaching or research institutions as the land-grant universities and experiment stations can usually call on other specialists for help. In such instances, the spectrograph and the photocolorimeter provide specific procedures for many determinations of both qualitative and quantitative tests that cannot be done in any other way. The delicate microassay of body fluids such as blood sera for vitamin content, or trace minerals depend upon a complete biochemical facility with personnel to perform the work. Such work is out of the question for the usual diagnostic laboratory, and would need to be referred to a laboratory able to do such work.

The majority of diagnostic laboratories can still perform a large number of qualitative or field tests which will indicate "yes" or "no" as far as the veterinary clinician is concerned (1, 2, 4, 5, 6, 7). The average veterinarian does not require the exactness and controlled procedures which are so necessary to the research worker. He can make the proper adjustment to correct the condition if he is able to determine whether the animal is probably deficient, within normal range, or possibly has an excess of such things as vitamin A, calcium, phosphorus, and magnesium. He has to be reasonably sure whether he is treating acute nitrite poisoning or prussic acid intoxication because it would make a difference in the antidote of choice (4).

The veterinarian needs to know whether or not he is treating an animal poisoned by lead instead of arsenic, or if both poisons are present. It may be an instance of antimony poisoning rather than either lead or arsenic. In ruminants, the enterotoxemia complex might appear to be a metallic intoxication. In horses, it might be poisoning due to a mold rather than a metallic intoxication. Such confusing pictures can be rapidly clarified by such a simple procedure as a Reinsch test which will rule the heavy metals (except lead) either in or out of the picture. A specific spot test for lead and mouse inoculation for enterotoxemia will eliminate or establish two more possibilities. Mold poisoning requires that one isolate the mold and reproduce poisoning with it, a long, and complex procedure.

In case a qualitative test is positive for a certain poison, and there are any possibilities for forensic procedures, one should refer the suspect materials to a commercial laboratory able to confirm the diagnostic laboratory's tests and if necessary, provide quantitative tests to support the diagnosis (10).

With the increased use of highly toxic insecticides, and many other industrial poisons as well as continued use of well known poisonous agents, the need for good field tests as well as laboratory toxicological procedures will increase. The more complete the laboratory facilities, the better the service which can be given. Although the fully equipped and staffed, complete
facility is the ideal it is the smaller, less elaborately equipped diagnostic laboratory which will have the greatest amount of toxicological as well as other work to do.

**Plate I**

Arsenic crystals, as seen in a positive Reinsch test for heavy metals are octahedral. Antimony crystals are very fine and needle like. The shape of the crystals should be confirmed by examination with the 100 X diameter magnification. This photomicrograph is 40 X.
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THE DIAGNOSIS OF LISTERIOSIS

JOHN W. OSEBOLD, D.V.M., PH.D.*

When your president, Doctor Sippel, asked me to discuss this subject I accepted because I have a research interest in this disease and am sure that you diagnosticians, working with field infections, can answer several of the questions relating to this world-wide zoonoses problem. As you know, the disease occurs in man in two principal forms just as it does in domestic animals, namely, meningo-encephalitis and genital infections. The latter are associated with abortions, stillbirths, and neonatal deaths. *Listeria monocytogenes* is receiving increasing study as an agent affecting human health. Case reports and discussions of listeriosis are appearing very frequently in current medical literature wherein such questions as the following are often asked: How many of these have we been overlooking up until now?—What can veterinary medicine tell us about the ecology of this disease and the modes of transmission to man? We probably ask ourselves similar questions or related ones. A question for us would be—do our laboratory records reflect the actual incidence of this problem? Two factors are concerned in the answer. The percentage of *Listeria* cases that reach the diagnostic laboratory is one factor. It is probably a very small percentage. Some experienced animal disease workers have thought that the greater percentage of clinical *Listeria* cases occur at low enough incidence rates to be accepted by the livestock producer as “normal loss.” Specimens probably get into the laboratory because of epizootics affecting greater numbers in a given population or because of occasional curiosity about the peculiar behavior of encephalitic cases. The other point relating to the validity of our records is concerned with the diagnostic methods used to reveal the infection when it is there. This is actually the topic for this discussion. My work at the present time is principally research and therefore the bulk of this data comes from experimental study. The subject can be considered under three headings: histopathology, bacteriological diagnosis, and serology.

HISTOPATHOLOGY

The tissue reactions of the central nervous system have been carefully described and need not be discussed in detail here (1). Suffice it to say that the lesions are quite characteristic although not diagnostic. Perivascular cuffing similar to that seen in several of the viral encephalitides occurs, but in addition there are nests of neutrophils and mononuclear cells in the brain

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parenchyma itself. Within these cell aggregates the bacterial organism may sometimes be observed. Pathologists occasionally see such lesions and anticipate a *Listeria* isolation only to receive a negative report from the bacteriology laboratory. This introduces the topic on isolation methods.

**BACTERIOLOGICAL DIAGNOSIS**

To establish a diagnosis we still feel obliged to isolate the agent. This is the most demanding of diagnostic procedures, and we should remember that it is a "far cry" from the diagnostic methods used in several other infections where we merely demonstrate the presence of an antibody or some reaction of hypersensitivity in the suspected host. This situation reflects the need for useful immunological procedures in the diagnosis of listeriosis so that we might gain information on true morbidity rates, rather than being content with isolating the organism from occasional fatal cases that reach our laboratories.

The bacteriological methods described here are those being used in our laboratory. They are not offered as the only methods or necessarily the best methods, but with the thought that some of our experiences in isolating this organism from tissue sites will be of interest. The specimens include either body fluids (such as cerebrospinal fluids and blood) or tissues from terminal cases. Numerous tissues may be examined depending upon the circumstances, but the liver, spleen, kidney, lung and brain are always included. Preferentially, brain stem should be cultured since the bacterium is concentrated in that area of the organ. A block of tissue about 1 cu. cm. in size is removed aseptically to tissue grinders, such as a mortar and pestle. Ample portions of the organ paste are streaked on blood agar plates, blood agar plates containing 0.05 percent potassium tellurite, and may also be inoculated into broth media such as tryptose broth or brain heart infusion broth. Broth media will be useful only if the specimen is free from contaminating microorganisms. The streaked plates offer the advantage of colony separation and aid the recognition of *Listeria* by demonstrating its hemolysin. The plate containing potassium tellurite will suppress many contaminants and will permit the growth of *Listeria* besides enterococci, *Corynebacteria*, and a few other organisms.

At the time original cultures are prepared, the remainder of each tissue specimen is set aside for reculture. The tissue paste is taken up in about 5 ml. of tryptose broth, placed in tubes, and held in the refrigerator (4°C) for six weeks; after which it is subjected to the same plating procedure. Reculture can, of course, be performed at periods less than six weeks. We have used six weeks because we find that all cultures likely to become positive will do so by that time. The importance of reculture may be seen in Table I, where 32 percent of 564 isolations were made only on reculture. The figures are even more striking if one examines the results from natural infections wherein 47 percent of the isolations were made only on reculture. Occasionally one encounters a situation where the organism is obtained originally and is lost by the time reculture is performed. Table I shows that this occurred only 11 times out of 575 isolations. When the reculture phenomenon was
first described it was related to bovine brain tissue (2), and this relationship has remained in the minds of many disease workers. Table II illustrates the reculture phenomenon from the tissues of four species, thus indicating that it is not restrictive as to host. This interesting trend in isolation has been encountered in a great variety of tissues and body fluids as shown in Table III where five tissues are listed. It is particularly significant that among the natural infections recorded in the table, there were only four isolations made originally from sites other than brain, and this total increased to 14 on reculture. Until reculture results have been examined, we take the view that \textit{L. monocytogenes} is not eliminated from the diagnosis in cases of encephalitis or genital infections.

\begin{table}
\centering
\caption{Isolations of \textit{Listeria Monocytogenes} Made by Dual Culturing}
\begin{tabular}{|l|c|c|c|}
\hline
 & \textbf{Experimental Infections} & \textbf{Natural Infections} & \textbf{Total} \\
\hline
Original Culture & 356 & 26 & 382 \\
Reculture & 515 & 49 & 564 \\
Percent revealed on reculture & 31\% & 47\% & 32\% \\
\hline
\end{tabular}
\end{table}

Total positive cultures = 564 obtained on reculture.
11 obtained on original culture only.

\begin{table}
\centering
\caption{Isolation of \textit{Listeria Monocytogenes} from Various Hosts Made by Dual Culturing}
\begin{tabular}{|l|c|c|c|}
\hline
 & \textbf{Number of Original Isolations} & \textbf{Number of Reculture Isolations} & \textbf{Percent Revealed on Reculture} \\
\hline
Sheep & 239 & 334 & 28\% \\
Cattle & 63 & 77 & 18\% \\
Rabbits & 78 & 148 & 47\% \\
Skunk & 2 & 5 & 60\% \\
Total Isolations & 382 & 564 & 32\% \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Isolation of \textit{Listeria Monocytogenes} from Various Tissues Made by Dual Culturing}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & \textbf{Experimental Infections} & & \textbf{Natural Infections} & \\
 & \textbf{Original} & \textbf{Reculture} & \textbf{Percent} & \textbf{Original} & \textbf{Reculture} & \textbf{Percent} \\
\hline
Brain & 93 & 106 & 12\% & 20 & 28 & 29\% \\
Liver & 30 & 47 & 36\% & 2 & 6 & 67\% \\
Spleen & 32 & 43 & 26\% & 1 & 3 & 67\% \\
Uterine exudate & 21 & 21 & 0\% & 0 & 3 & 100\% \\
Kidney & 20 & 32 & 38\% & 1 & 2 & 50\% \\
\hline
\end{tabular}
\end{table}

Cultures are incubated at 37\degree C. and observed daily for characteristic colonies for five days. The procedure for body fluid cultures is the same as for organ cultures including the reculture examination. When \textit{Listeria-like}
THE DIAGNOSIS OF LISTERIOSIS

organisms are encountered, they are subjected to the following identification procedures. Spot agglutination tests are performed on a slide employing anti-
Listeria serum (beware of autoagglutination by certain diphtheroids). The organism is also inoculated into tryptose broth where Listeria will produce uniform turbidity in contrast to many Corynebacteria which produce granular growth or no growth unless serum is added. If the organism passes these tests, it is inoculated into a battery of differential carbohydrates (See Bergey's Manual). Two animal inoculation procedures are also used. Four or five mice are inoculated subcutaneously with varying dilutions of a 24-hour tryptose broth culture starting at about 0.5 ml. and decreasing to 0.001 ml. Deaths usually occur three to 10 days following inoculation, and autopsy reveals the characteristic multiple necrotic foci in liver and spleen. The second animal test demonstrates a characteristic keratoconjunctivitis in the eye of a rabbit or guinea pig. It is performed by gently massaging a cotton swab dipped in broth culture against the conjunctiva. Purulent reaction appears within 24 hours and is well advanced at 48 hours along with opacity of the cornea.

SEROLOGY

Serological methods have not yet been developed adequately for routine application in the diagnosis of listeriosis. Agglutination tests can be used and are valuable in research. The procedure has a serious limitation for use in the diagnosis of natural infections because the sera of man and many species of animals normally contain antibodies which will react with Listeria antigens (3). This means that the majority of sera will have some level of agglutinating antibody titer. Unless paired sera are available and a marked shift in titer occurs, the agglutination test becomes uninterpretable. In research, a base line of normal reaction can be determined for each animal, and antibody response above that level becomes meaningful.

Complement-fixation tests are under study and there is reason to think that this method will circumvent the disadvantages encountered with the agglutination reaction. The research intent is to avoid denaturing procedures in antigen preparation so that the entire antigenic mosaic of the organism can participate in the fixation of complement. Antibody populations in immune serum would then quantitatively override the importance of "normal" antibodies, which appear to combine with polysaccharides on the bacterial cell surface. Complement-fixation tests are not, as yet, receiving wide application although it may be assumed that this will become a very useful immunological procedure.

A third serological procedure, which has been developed in our laboratory, offers promise for differentiating sera that contain only the confusing "normal" antibodies from those which contain true immune antibodies as well. It is called the Antigen-Fixation Test and will be described in detail elsewhere. Briefly, the test depends upon the firmness of fixation of hematoxylin stained antigen to a strip of filter paper following its contact with the test serum. The edge of the filter paper strip is immersed in a solvent
which migrates up the paper. As it encounters the area of antigen-antibody reaction, it will wash away part of the stained antigen in sera containing only "normal" antibodies. Sera containing immune antibodies will firmly fix the antigen on the paper so that the washing effect does not occur. The test is simple to perform, and it is hoped that it will have a useful place in the diagnostic laboratory.

DISCUSSION

I would like to urge diligent search for this organism in the following situations. Encephalitis cases should receive careful study. There is reason to think that many negative rabies reports may actually be undetected *Listeria* infections. When brain tissues are submitted for rabies examination, perhaps a portion of the brain stem could routinely be subjected to bacteriological study. In all cases of abortion this agent should be suspected. We have recently disclosed the infection in three herds of cattle in California which have had a persistent abortion problem. The pathologist should watch for this organism as a cause of valvular endocarditis. The bacterium is known to produce clinical endocarditis in man and we have encountered well-formed valvular lesions in three experimental sheep. *Listeria monocytogenes* has been isolated from the milk of naturally infected cattle and goats. This is being considered as a possible epidemiological link for infection in man. Bacteriological examinations of milk samples should include this organism among those anticipated.

Regarding the listeriosis problem in man, one can see an analogy to brucellosis wherein the medical profession relied very heavily on the veterinary profession to outline the total problem and locate man's place in the infection chain. I would like to close by quoting from a reprint regarding the diagnosis of listeriosis in man: "The CDC will accept cultures for identification, or perhaps you will find a college of veterinary medicine in your area is interested in the problem and able to assist you" (4). I think that we are interested and that we can assist them.

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LABORATORY DIAGNOSIS OF ANTHRAX


A positive diagnosis of anthrax can be made only by laboratory procedures. It is, therefore, imperative that the specimen submitted to the laboratory for diagnosis be properly collected, identified, and prepared for shipment. Specimens collected under proper conditions will enable the laboratory to give the earliest possible definite diagnosis. This is most helpful in preventing spread of the disease, and failure to properly diagnose the case may prove disastrous.

When blood and tissue specimens are fresh, properly collected, and free of antibiotics, there is no difficulty in isolating Bacillus anthracis. Decomposed or contaminated specimens are likely to yield anthrax-like organisms, and destruction of the anthrax organisms may occur due to putrefactive processes or the presence of other organisms.

Improper collection and handling of the specimen may result in contamination with other organisms such as Pasteurella, Clostridium, Streptococcus, Staphylococcus or Pseudomonas.

The inoculation of pathogenic contaminants into laboratory animals may cause early death, or may delay the onset of anthrax beyond the normal seventy-two hours. Careful studies are necessary with contaminated materials to avoid an erroneous diagnosis.

One of the most satisfactory methods for the collection of specimens from animals dying of anthrax is the application of three to five drops of blood to a piece of sterile umbilical tape three to five inches in length which has previously been placed in a sterile stoppered test tube such as a rubber stoppered bleeding vial. The blood should be collected by means of a sterile syringe from the jugular vein of the animal suspected of dying from anthrax. Approximately one-half inch of the tape should be allowed to extend beyond the stopper, and several specimens should be prepared from each case. The specimen should be taken to the office and allowed to stand with the stopper removed to allow the blood to dry on the tape. This usually requires two to three hours. The tubes should then be stoppered, properly identified, carefully wrapped in paper or cotton to prevent breakage, and then placed in a double container. A metal mailing tube is preferred for shipment to the laboratory, and the history and other pertinent information should accompany the specimen. Refrigeration is not required for properly dried specimens.

If umbilical tape is not available, the method described by Stein (15) may be utilized to advantage. This method consists of a sterile cotton swab attached to a cork and placed in a small sterile vial. The cotton swab should

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be only partially soaked in order that the swab will dry. The most common error encountered by this method is complete soaking of the swab, which results in incomplete drying and growth of other organisms.

If neither of the above are available, the lower ear of the dead animal may be removed and forwarded as a specimen although experience has proven that this method is in many instances, unsatisfactory. It is essential that this specimen be submitted under refrigeration.

If a necropsy is performed, specimens may be obtained directly from a spleen on a sterile cotton swab. If a sample of the spleen is to be forwarded, it may be held in the gloved hand and the glove turned inside out, leaving the tissue inside the glove. The glove opening should be securely tied. If samples are submitted in this manner, they should be sent under refrigeration. Experience has indicated that swabs or umbilical tape samples are more satisfactory than wet specimens such as the spleen or tubes of blood from a carcass. It should be remembered that there is a greater danger in submitting wet specimens such as a piece of spleen or lymph node because of the possibility of breakage of the specimen container. Also, personnel handling the sample may become exposed.

In case of localized swelling or edema, the organism may be restricted to the area of swelling. The best procedure for the collection of a specimen from such an area is by means of a sterile needle (three inch, 14 gauge) inserted into the swelling. The aspirated material should be placed on umbilical tape or a cotton swab.

In swine, anthrax is most often a localized infection of the pharyngeal region. Because of this, cervical lymph glands are more likely to contain organisms than is the blood or other tissue specimens. If lymph glands are shipped, they should be either iced or packed in borax. The package should be properly labeled and the laboratory should receive pertinent information including treatment with antibiotics or other drugs. Antibiotics affect not only the length of time required to produce lesions and death in experimental animals, but may also affect the morphological characteristics of the organism or the colonies. In addition, information should be submitted if the carcass is decomposed or contaminated in order that the specimen may be properly prepared for inoculation and culture.

Speed in the laboratory diagnosis of anthrax is an important factor in the control of outbreaks. Since *B. anthracis* morphologically closely resembles several other organisms in the Bacillus group which are considered non-pathogenic, it is hazardous to base a diagnosis on the presence of rod-shaped organisms in blood smears. Other criteria must be considered in making a diagnosis of anthrax. The procedures outlined as follows should enable the laboratory to determine the presence or absence of anthrax with little or no possibility of error.

Add two to three ml. of sterile saline to tube containing swab and agitate. Prepare smear from this suspension, heat fix, Gram stain, and examine. *Bacillus anthracis* appears as a straight, square-ended Gram positive rod in chains, end to end, or sometimes occurring singly and in pairs. The faint outline of capsules may also be noted. Smears prepared from dry material
often show organisms with small, oval-shaped spores. All in between variations occur depending on moisture content, time elapsed since the death of animal, and shipment time.

With a sterile loop inoculate a loop full of the saline suspension on two nutrient agar plates. Streak for isolated colonies. Place remainder of saline suspension in water bath at 80°C for ten minutes. This will materially reduce the number of non-spore-forming contaminating organisms in the specimen. Some contaminating organisms tend to inhibit the growth of B. anthracis. Inoculate the heated suspension on two nutrient agar plates as above. This pasteurization process is intended both to heat-shock any spores present and to remove skin, dust, water, and post-mortem contaminants. Fresh specimens may not contain spores of B. anthracis and, therefore, may be negative following heat treatment.

As B. anthracis bacteriophage is a reliable means of differentiation of the pathogenic from other aerobic spore-forming organisms, it may be employed on the first attempts to isolate anthrax organisms. The site where a drop of phage solution has been dropped should be marked on the glass of the petri dish by an appropriate ring. Errors due to only a few viable organisms should be avoided in the interpretation of phage activity.

Colonies of B. anthracis are quite characteristic in appearance after 18 to 24 hours incubation. They are small and irregular in shape and may show common shaped projections from the main body of the colony. They are greyish-white, flat, with a ground glass appearance and a friable consistency, stringing like warm butter on the loop. Observation of the colony under low power magnification will reveal the characteristic medusa-head arrangement of the bacterial filaments. Positive plates treated with bacteriophage will show a clear zone due to lytic action in the area where the phage was placed, provided there is no overgrowth with contaminating organisms.

A typical colony should be transferred to nutrient agar slant for use in further tests or as a stock culture. From an isolated characteristic colony inoculate semi-solid gelatin media. Stab the medium to approximately one inch. Incubate 18 to 24 hours. B. anthracis is non-motile and produces on a gelatin type semi-solid agar an “inverted pine tree” like growth.

Inoculate infusion broth and examine at end of 18 to 24 hours. Anthrax produces little or no turbidity, the growth resembling a loose ball of cotton in the bottom of the tube. This is characteristic of anthrax organisms, but Bacillus mycoides has identical characteristics, and variant forms resembling B. anthracis have been observed.

Specific bacteriophage typing may be accomplished by the use of a light saline suspension of B. anthracis from the nutrient agar slant. Transfer a loop of the suspension to an agar plate and spread over the plate by means of a sterile bent glass rod. Allow the plate to dry by incubating for approximately two hours. Place one drop of the phage in center of plate and incubate plate for 18 to 24 hours. If the organism in question is B. anthracis the area where the phage was placed will be essentially clear while a profuse growth is present on the remainder of the plate. In some instances the phage area will not be entirely free of growth. This may be
due to an overwhelming inoculum or other factors unknown. To our knowledge no proven strain of \textit{B. anthracis} has failed to be lysed by the Cherry bacteriophage and no other Bacillus spp. is subject to the lytic action of such phage.

As pathogenicity is a reliable and necessary factor in the identification of anthrax, at least the first losses should be identified by animal inoculation and pathogenicity studies.

Inoculate subcutaneously 0.2 to 0.3 ml. of a saline suspension from plate and/or slant into two guinea pigs. If anthrax is present the guinea pigs usually die in 24 to 96 hours.

Observe for characteristic lesions such as subcutaneous edema at site of inoculation, dark colored, unclotted blood and swollen spleen. Spleen and heart blood should be cultured. A drop of blood from the heart is streaked onto nutrient agar. Also inoculate the cut surface of the spleen on an agar plate by imprint and streak for isolated colonies from the imprint.

Prepare and stain with methylene blue smears from heart blood and spleen of dead guinea pigs. Observe for characteristic organisms and the presence of capsule haloes.

Examine plates after 18 to 24 hours incubation for typical colonies and identify same as outlined above with one or more of the confirmative tests, especially motility and phage activity. When a laboratory encounters anthrax in field specimens it may be well to renew \textit{B. anthracis} phage cultures and to check its potency. Inoculate a flask of nutrient broth with an actively growing virulent strain of \textit{B. anthracis}. Incubate for eight to 12 hours and add several ml. of the phage virus suspension. Replace in incubator and allow to grow for an additional 18 to 24 hours. Filter the culture through a Seitz or other bacterial filter to remove \textit{B. anthracis} organisms. The filtrate containing the virus should then be tested for sterility by the usual tests. Should the filtrate not be potent enough, repeat the procedure using a larger inoculum of the virus suspension previously prepared and \textit{B. anthracis} culture. The phage should be tested on known \textit{B. anthracis} cultures and evaluate as to potency (3).

\section*{Isolation of Bacillus Anthracis from Animal By-Products}

A search of available literature in quest of detailed comparative studies evaluating the efficacy of various procedures recommended for the isolation of \textit{B. anthracis} from such products as wool, hides, and bone meal has met with a remarkable lack of success. The relatively small number of published reports on this subject and particularly the complete absence of extensive evaluations poses a difficult problem for the investigator and makes any attempt to review critically and recommend specific techniques, with adequate assurance of their reliability, an almost impossible task. The isolation of \textit{B. anthracis} from animal products is difficult due to contamination with other spore formers and certain ubiquitous, rapidly growing bacteria, such as the pseudomonads and Proteus species, which may be antagonistic to or overgrow the anthrax organism.
Preliminary treatment of materials to be examined is essential to remove gross particulate matter, to destroy as large a proportion of contaminants as possible and, in some instances, to concentrate the number of *B. anthracis* spores present. The following preparatory procedures have been recommended:

A. Soaking of hair or wool in distilled water or weak KOH solution for 4 hours (1).

B. Preparation of approximately 10 to 20 percent suspension in distilled water or saline, grinding in a mortar, filtration through gauze, or allowing to settle at room temperature (4, 6, 8, 13).

C. Treatment of a saline suspension of material with one to two percent phenol for one hour (9).

In all instances except the latter, the supernate is heated to destroy vegetative cells before either culturing or inoculation of animals. The recommended time and temperature for the heat “shocking” varies with different authors. Since little additional killing of contaminants is accomplished by temperatures above 60-65°C, and some strains of anthrax spores are not completely resistant to 70 to 80°C (8), it is recommended that 60°C for 20-30 minutes be used. This procedure appears to enhance germination of spores also.

After preparation and heating of suspensions, the material is then examined for the presence of the anthrax organisms by cultural and animal inoculation procedures. Some authors recommend centrifugation at 2000-3000 RPM's for 20-30 minutes and the examination of the sediment thus obtained. It should be noted that materials prepared by treatment with one to two percent phenol, should not be used for animal inoculation unless the phenol is removed.

It is recommended that both direct culture and animal inoculation procedures be used. Several workers have also had some success with the membrane filter technique (4).

**Direct Culture:** Several aliquots of the heated sample in 0.2 to 0.3 ml volumes are streaked on plates of freshly prepared nutrient and/or blood agar. The usual nutrient, infusion, or tryptose agar culture media are quite satisfactory for the growth of *B. anthracis*. Blood agar prepared by addition of five percent sheep or rabbit blood to an appropriate basal medium is recommended since some differentiation on the basis of both colonial morphology and hemolysis can then be made on the initial plates. Four plates to which may be added varying amounts of the suspension (up to two ml) have been used by some workers. Two selective media for isolation of *B. anthracis* have been described (13, 14), and a preliminary report describing the use of one of these media for isolation of anthrax from soil samples was published in 1955 (12). On the basis of the reports of their originators, these media would seem to deserve serious consideration when used under the specific conditions described by the authors. Until these media are more widely employed and critically evaluated, it is recommended that their use be in addition to, rather than as a substitute for, nutrient and blood agar.
Animal Inoculation: Both mice and guinea pigs have been used and there is no general agreement as to which species is to be preferred. In the opinion of the writer, and others, the guinea pig offers several advantages. Larger volumes may be inoculated with impunity into a single animal, which is especially advantageous if the original material contains relatively few spores. The guinea pig appears to be less susceptible than the mouse to toxic effects of nonspecific substances in the materials to be examined, and is apparently less likely to succumb to infection with other aerobic spore-forming contaminants. Again, it has not been possible to find reports of comparative studies using guinea pigs and mice with the same materials. Consequently, this opinion is based on the observations that workers using mice report a relatively large proportion of nonspecific deaths, whereas those using guinea pigs have reported fewer.

Several animals are inoculated with 0.5 to 1.0 ml. aliquots of the heat-shocked fluids, prepared as outlined above, by either the subcutaneous or intramuscular route.

For putrid or highly contaminated materials the authors of Topley and Wilson (16) have recommended inoculation by the scratch technique. To eliminate infection and deaths from the commonly found anaerobic spore-formers, the animals may be passively immunized 24 hours prior to inoculation with clostridial antitoxins. Recommendations from reference given: 1000 units Cl. welchii, 1000 units Cl. oedematiens, and 500 units Cl. tetani antitoxins.

Laboratory animals infected with anthrax most usually die within 48 hours. Both earlier and later deaths are frequently nonspecific, although some deaths from anthrax in mice or guinea pigs may occur in 24 to 36 hours, and as late as four to five days. In any event, all animals should be autopsied and examined thoroughly for characteristic gross changes, and for demonstration of the specific organism both by smears of blood and tissues, and isolation on culture media.

The guinea pig dead of anthrax will typically show gelatinous, frequently blood-stained, edema at the site of inoculation. Blood will be dark-red and remain unclotted to give a mushy consistency. The spleen is usually dark red and enlarged. Capsulated organisms of typical morphology can be demonstrated microscopically in blood films or tissue imprint preparations appropriately stained. B. anthracis can be isolated without difficulty also from blood, spleen, etc.

The specific differentiation of B. anthracis from other anthrax-like aerobic spore-formers may present problems. Suggested differential characteristics have been described by various authors (2, 5, 7, 10). In all cases, it has been repeatedly pointed out that no single test should be relied upon.

In our experience, the following combination has been most helpful in identification of B. anthracis:

1. Characteristic colonial morphology on nutrient and blood agar.
2. Slight or no hemolysis within 24 hours on blood agar.
3. Susceptibility to lysis by specific bacteriophage.
LABORATORY DIAGNOSIS OF ANTHRAX

In addition to the above, Ivanovics and Foldes (7) have demonstrated that phosphatase production (a test with which we have, as yet, had no experience using the group of organisms) is also a reliable differential characteristic. These authors believe that the combination of three reactions: (a) Phosphatase production, (b) penicillin sensitivity, and (c) susceptibility to action of specific bacteriophage, will clearly differentiate \textit{B. anthracis} from \textit{B. cereus}. This paper of Ivanovics and Foldes is available in English, and is especially recommended for its discussion of the problems related to the identification and taxonomic relationships of \textit{B. anthracis}.

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LEPTOSPIROSIS DIAGNOSIS—PRESENT AND FUTURE

ERSKINE V. MORSE, D.V.M., PH.D.

A brief history of leptospirosis diagnosis and testing agencies was given. The discussion was limited to the leptospirosis problem as it pertains to livestock in the United States.

Two distinct needs are evident: first, tests or procedures for the diagnosis of the acute or clinical outbreak and second, practical means of detecting the chronic-phase carrier. The solving of these two problems will lead to effective control programs and eventually the accreditation status desired by the livestockman. These needs, particularly the latter have led to great numbers of serum samples being sent to laboratories.

The status of a herd or an individual cannot be ascertained by just one test or a single herd test. The clinical history is equally as important as serological findings. The “paired sample” technique is advised but often impractical. A definitive diagnosis can be made only by isolation of the infecting agent. Such is generally “out of the question” for most veterinary diagnostic laboratories or the practitioners whom they serve.

It was recommended that:

1. Work continue with adequate support to ascertain the most practical and reliable diagnostic test for leptospirosis.
2. Existing test procedures, i.e. Stoenner or Galton plate tests, etc. which have been standardized should not be modified. Obviously, this is not meant to discourage experimentation, but modified techniques should not be put into practice until they have been assayed critically.
3. Some standard serological test for leptospirosis is needed for veterinary diagnostic laboratories. It would appear that the agglutination-lysis tests should be the choice. The United States Livestock Sanitary Association’s Leptospirosis Committee is preparing protocol for such a standard test. It is hoped that laboratories will adopt this procedure and utilize it as a reference test. In order to ascertain relative, and by no means fixed, costs of an agglutination-lysis test laboratory or a plate agglutination test laboratory the following information was presented:

† Associate Director, Veterinary Medical Research Institute, Iowa State University of Science and Technology, Ames, Iowa. Published as paper N. S. 601, Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University of Science and Technology.
# Leptospirosis Diagnosis—Present and Future

## Table 1
**Costs for Rapid or Plate Agglutination Diagnostic Laboratory***

<table>
<thead>
<tr>
<th><strong>Staff:</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostician-supervisor, 1/2 time</td>
<td>$850</td>
</tr>
<tr>
<td>Technician, full time</td>
<td>4,000</td>
</tr>
<tr>
<td>Clerk, 1/2 time</td>
<td>1,500</td>
</tr>
</tbody>
</table>

**Equipment:**

<table>
<thead>
<tr>
<th>Item</th>
<th>1st Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
<td>1,500</td>
</tr>
</tbody>
</table>

**Supplies:**

<table>
<thead>
<tr>
<th>Item</th>
<th>1st Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racks, glassware, pipettes, etc.</td>
<td>500†</td>
</tr>
<tr>
<td>View boxes</td>
<td>100</td>
</tr>
<tr>
<td>Commercial antigens</td>
<td>4,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Total:</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First year total</td>
<td>$12,450</td>
</tr>
<tr>
<td>Succeeding years</td>
<td>10,700</td>
</tr>
</tbody>
</table>

* To process 5,000 to 10,000 samples per month.
† Repetitive at $250 per year.

## Table 2
**Costs for an Agglutination-Lysis Diagnostic Laboratory***

<table>
<thead>
<tr>
<th><strong>Staff:</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostician-supervisor, 1/4 time</td>
<td>$2,125</td>
</tr>
<tr>
<td>Technicians—2 full time</td>
<td>8,000</td>
</tr>
<tr>
<td>Laboratory glassware washer—full time</td>
<td>3,000</td>
</tr>
<tr>
<td>Clerk, 1/2 time</td>
<td>1,500</td>
</tr>
</tbody>
</table>

**Equipment:**

<table>
<thead>
<tr>
<th>Item</th>
<th>1st Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darkfield microscope, illuminator, etc.</td>
<td>1,200</td>
</tr>
<tr>
<td>Incubators, water baths, centrifuge, filters, etc.</td>
<td>3,500</td>
</tr>
<tr>
<td>Hood or sterile cubicle</td>
<td>1,500</td>
</tr>
</tbody>
</table>

**Supplies:**

<table>
<thead>
<tr>
<th>Item</th>
<th>1st Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racks, tubes, pipettes, slides, etc.</td>
<td>2,400†</td>
</tr>
<tr>
<td>Chemicals, media, sera</td>
<td>600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Total:</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First year total</td>
<td>$23,825</td>
</tr>
<tr>
<td>Succeeding years total</td>
<td>16,225</td>
</tr>
</tbody>
</table>

* To process 5,000 to 7,000 samples per month.
One technician = 3,000 samples per month.
† Repetitive at $1,000 per year.
LABORATORY NOTES ON THE DIAGNOSIS OF
ANAEROBIC BACTERIAL DISEASES

COMMITTEE ON ANAEROBIC BACTERIA

EDWIN M. ELLIS, Chairman, D.V.M., PH.D., E. M. BALDWIN, D.V.M., M.S.,
L. H. GRINNER, D.V.M., M.S., PH.D., L. R. VAWTER, D.V.M., M.S.

INTRODUCTION

It is not the purpose of this Committee to present a complete key to the
morphological and cultural characteristics of the anaerobic bacteria since
this subject is readily available in the current literature, but, rather, we have
gathered together helpful information for use by veterinary diagnostic
laboratories confronted with the task of diagnosing diseases caused by
anaerobic bacteria.

It is our purpose to cover only those anaerobes that are of more common
occurrence and frequently associated with animal diseases. Anaerobic bacteria
should be identified as promptly as possible in order that treatment, control,
or preventative measures can be instituted to suppress the disease. Proper
selection and handling of tissues and exudates is most important. It is the
duty of each laboratory to instruct those it serves in this matter. Collection
of tissues, organs and body fluids from decomposing carcasses is to be
avoided if possible. Bacterial invaders quickly enter most tissues and cause
much delay in the laboratory. More latitude is allowed in cold weather.

It is not possible in an outline of this type to include stepwise all of the
procedures that at some time might be necessary for the isolation of every
anaerobe. To make certain that every laboratory is able to isolate and
identify the more common anaerobic species, this work has been undertaken.
Therefore, it is hoped that if the following points are observed, any laboratory
should be able to handle at least the five more common species. Though it
is true that many of the techniques are found in the literature, it is nonetheless
important that they be gathered into one publication consisting of the
experiences of several outstanding workers in this segment of bacteriology.

SHIPPING INSTRUCTIONS

Each laboratory has its own recommendations for shipping tissues to the
laboratory. One of the most successful and popular is the use of a thermos
cooler. Tissues or exudates are placed in individual plastic bags and tied
tightly. These are then placed in the cooler which has been filled with cracked
ice. Regardless of the method of shipment, it is important that the intestines
or intestinal material is sent in a separate container and does not become
associated with the tissues or organs involved.

Blood should be placed in sterile tubes and refrigerated with cans of frozen
water. Bang’s tubes with punctured stoppers should not be used, as they
frequently leak. Sterile swabs in tubes are useful for exudates or blood. It is helpful, if possible, to chill or freeze specimens intended for culturing prior to shipment. Tissue specimens for culture must be large enough for practical handling. Organ sections should be a minimum of three inches square and organ thickness. Both blood and tissue is desirable as *Cl. septicum* and *Cl. feseri (chauvei)* may be present in blood, but *Cl. hemalyticum* and *Cl. novyi* may not.

**HISTORY**

In order that the proper diagnostic methods may be employed quickly, the following points should be taken into consideration:

a. Number of animals in herd.

b. Species of animals.

c. Number of animals affected.

d. Number of deaths.

e. Records of vaccinations.

f. Management practices.

g. Clinical signs.

h. Necropsy findings.

i. Interval between death of animal and necropsy.

j. Condition of specimens at time of arrival at laboratory.

k. Any other pertinent information that the attending veterinarian can supply.

**ANAEROBIC METHODS**

The laboratory should adopt a practical and workable method for the culture of anaerobes; this method should insure strict anaerobic conditions. Many methods have been described in the literature, and each laboratory should select the method that is most adaptable to its particular needs. One of the older methods is the use of an anaerobic jar and pyrogallic acid with sodium hydroxide. Ten gms. of pyrogallic acid in 100 ml. of 10 percent sodium hydroxide per quart capacity are used. The disadvantage of this method should be kept in mind in order to assure good results. It should be made certain that an excess of alkali is added to the pyrogallic acid to insure complete neutralization. In this case, all of the oxygen is removed and anaerobic conditions are produced. However, this action also absorbs carbon dioxide which is required by some microorganisms for initial growth from a small inoculum. In addition, it is known that alkaline pyrogallate produces a small amount of carbon monoxide, which can be somewhat inhibitive.

Perhaps a more satisfactory method of producing anaerobic conditions is the use of a dessicator jar with a single rotatable outlet. The chamber is repeatedly evacuated of air and the vacuum is replaced by nitrogen. A slight vacuum should remain after the last filling. If water-washed nitrogen is used, the nitrogen may contain some oxygen. This gas can be removed by passing the nitrogen through a bottle containing alkaline pyrogallate. It is
always well to use a methylene blue indicator tube in the chamber as an indicator of anaerobic conditions. Directions for preparing such a tube are listed in the appendix.

EXAMINATION OF TISSUES

Tissues received for anaerobic culture should be examined and the following noted: Gross appearance, color, odor, and whether there is emphysema. Smears should be prepared from each piece of tissue. A smear should be prepared from each fluid or swab presented. A Gram stain and a spore stain should be routine practice. It is important that 95 percent alcohol be used as a destaining agent. The smear should be examined for microorganisms, noting staining reaction, morphology, presence or absence of spores and the numbers of organisms present should be estimated.

DISEASES

The diseases caused by anaerobes can be divided into two categories: (a) Infectious diseases—diseases in which the organisms invade and reproduce in the tissues of the host, (b) non-infectious diseases—enterotoxemias. We will consider first the infectious diseases caused by anaerobes.

CULTURING OF INFECTIOUS CLOSTRIDIA

In culturing, it is suggested that several isolations be made and compared as to morphology, motility, and biochemical reactions. The specimens for culture should be handled carefully. If it is deemed necessary, the specimen may be dipped in boiling water for a minute or two to kill outside contaminants, or the surface of the tissue may be seared with a hot spatula or iron and then incised with a sterile knife. It is generally necessary that one of these procedures be carried out in order that contaminating bacteria are removed insofar as possible. Either snip out liberal pieces of tissue for direct culture or prepare a suspension by grinding with sterile sand in a mortar or immulsify in a Waring blender with either saline solution or infusion broth. For primary isolation of anaerobes suitable media should be inoculated with a tissue suspension, blood, or exudate, in the event that gas edema infections, i.e., Cl. jesseri, Cl. septicum, Cl. novyi, Cl. sordellii or Cl. tetani are present. As all of the important clostridial pathogens sporulate to a greater or lesser extent, the irrelevant aerobes, excepting enterococci and sporulating aerobes, can be logically eliminated by heating some of the tubes of the primary suspension at 80 to 85° C. in a water bath for intervals varying from 10 to 25 minutes. Periods as short as 10 minutes have been used successfully by some workers and periods from 20 to 25 minutes have been reported equally successful. It is possible, however, that if only vegetative forms are present, these may be killed by heating in such a manner. It is always well to inoculate media with heated and unheated cultures. In addition, if a rapid diagnosis is necessary, a guinea pig may be inoculated intermuscularly with the unheated preparation. This procedure, besides providing information for a tentative diagnosis, aids in determining pathogenicity and provides, in
LAB. NOTES ON ANAEROBIC BACTERIAL DISEASES

many cases, a pure culture for further study. Further information regarding
animal inoculation will be found in a later section.

Most of the pathogenic species will show vigorous growth under favorable
conditions in 18-24 hours in the routine culture media listed in the appendix.
Many show some sedimentation in 24 hours. Some cultures of *Clostridium feseri* do
not show much growth or gas until after 24-36 hours incubation. *Clostridium feseri*,
*Cl. septicum* and *Cl. boulinum* may sporulate in the first 24 hours. Sporulation
is poor by *Clostridium hemolyticum* in most media, but occurs quite abundantly in
the lesions in cattle. Resistance to heat is variable, but most strains will not
tolerate heating above 85°C.

GENERAL PROCEDURES

A Gram stain of the smears of all materials should be made. All tissues
and exudates presented should be examined in this manner. Tissue sus-
pensions as already described can then be strained through several layers
of sterile gauze. The tissue suspensions thus obtained can now be inoculated
into the proper media. Media listed in the appendix can be used and the
choice is much a matter of preference depending upon whether one is in-
terested in primary isolation or biochemical reactions of an already isolated
organism.

It is always safer to make several isolations and compare them as to
morphology, motility, and biochemical reactions (see Smith (6), or Willis
and Hobbs (10, 11) for morphological descriptions and biochemical methods,
and Reed and Orr (5) for culture media). Unheated tissue suspensions may
contain pure cultures of anaerobic bacteria, but more often are contaminated.

Incubation time will vary, but after growth is observed cultures should be
examined and smears prepared for Gram’s stains. All isolates should be
compared to be certain that only one species of Clostridia is present.

Willis (10) has suggested a rapid method for purification of some Clostridia
from mixtures with other organisms, especially the aerobic spore formers by
inoculation into deep glucose broth containing 1 mg. per ml. of streptomycin
sulfate. Willis & Hobbs (11) have recently found that the saccharolytic and
proteolytic properties of Clostridia can be more efficiently demonstrated by
the use of glucose-gelatin and lactose-egg-yolk-milk agar. They also showed
that 250 mcg/ml. of neomycin acts as a selective agent for *Clostridium perfringens*,
Type A.

Pure cultures are absolutely essential for complete identification of the
suspected pathogens. This requires appropriate culture media and methods.
Several methods have been devised for cultivation of anaerobic bacteria,
but some of them are not always entirely satisfactory for fastidious species.
The use of Brewer and McIntosh-Fildes anaerobic jars, the cromium sulfuric
acid method of Rosenthal, and deep agar dilution culture methods are
described by Smith (6).

The deep agar dilution method of Hesse for obtaining isolated colonies by
successive dilution in a series of agar tubes is one of the oldest and best
methods of obtaining pure cultures of anaerobic bacteria. It can be used
in any laboratory. The basal medium used for making the agar must be adequate for growth of fastidious species. Fresh beef liver infusion or the digest types are generally satisfactory basal media and should have an agar content of 1.5 to 2 percent. It must be clear so that the colony structure can be easily observed. These media may also be used as plating media. The addition of 0.1 percent of glucose is desirable for good colony growth but 0.2 to 0.3 percent of glucose is better for Cl. feseri.

As a general rule, deep agar in tubes used for dilution shake cultures should be not over one to two weeks old at most for fastidious species like Cl. hemolyticum and Cl. novyi. It should be stored in a refrigerator in cans with tight lids. Absorbed air in the agar tubes should be expelled by boiling for 10 minutes in a water bath before inoculation.

Inoculation should be made at once after cooling to 45-50°C. The tubes should then be rolled between the palms of the hands and placed in cold water at once for a few minutes for hardening before incubation. An uninoculated control tube containing a drop of methylene blue serves as an index of anaerobiosis of the medium used (see formula in appendix).

Most of the obligate anaerobic species develop colonies in the agar column at least 1 cm. below the surface usually in 24 hours incubation, but some species may not develop full colonial features for two or three days, i.e. Cl. feseri and Cl. botulinum. Most species usually show a characteristic colony type but at times a single species may exhibit variable colony features. They usually appear at first as small lenticular types, but in six to 12 hours develop into forms more or less characteristic of the species. These may be lenticular, multiplante discs, bursting grenades, dense chestnut burr with short filaments, loose wooly filamentous, or rhizoid types. Recognition of colonial types of the various species comes only with experience. Description of colonial types and features of individual species under various cultural conditions is too extensive to describe in this paper, but is given in journal articles and textbooks dealing with individual species and cited in the bibliography.

It is advisable to make two or three passages through deep agar dilution shake cultures or by plating methods with single colony isolates. This is mandatory when the original material contained a mixed flora of sporulating anaerobes forming rhizoid or spreading filamentous colonies. The plate colonies can only be studied by use of plate cultures in anaerobic jars or in the Bray and the Spray dishes or with the Brewer plate method. These methods are most desirable for determining the surface colony features and behavior on blood agar and egg yolk agar plates.

Once pure cultures are obtained, detailed study of the biochemical features may be started. Incubation should be continued from two to seven days or more to observe the slow reactions and proteolytic features of some species. Most of the cultural features are developed sufficiently in two or three days to get a presumptive idea of the identity of the culture at hand. The various types of fermentable carbohydrate and other types which would identify the individual species should be included. Some features may be determined incidental to isolation procedure.
The chart given by Merchant (2) gives the important features of the pathogenic anaerobes encountered in veterinary bacteriology. Smith has a more extensive chart of the species of Clostridia including many non-pathogens. Both of them are dependable sources of information.

A modified formula of the basal medium devised by Reed and Orr (5) has proved quite satisfactory for carbohydrate fermentation features provided proper technique is used.

**ANIMAL INOCULATION**

The following animals are useful in determining pathogenicity, which is an important aid in diagnosis: Guinea pigs, mice, rabbits, hamsters, and pigeons. Inoculations may be IV, IP, or IM, depending upon the species isolated. The following should be considered upon death of the laboratory animal:

1. Consider the time lapse from inoculation to death.
2. Conduct a complete necropsy and note type and distribution of lesions.
3. Prepare smears of muscles or impression smears from surfaces of viscera, especially liver, and examine for organisms and compare with isolates from original tissue. This procedure may serve to differentiate between *Cl. feseri* and *Cl. septicum*.

Specific antisera may be necessary for mouse inoculation in the case of the enterotoxemias. These would include neutralization or protection tests in mice and possibly other animals. The reaction of laboratory animals to the various species of Clostridia is discussed in the following paragraphs.

*Cl. feseri* produces quite typical dry, dark red gaseous lesions in thigh muscles of guinea pigs with 0.1 to 0.5 mls. of 36 to 48 hour cooked meat medium culture. The lesion fluid in cattle is very lethal and 0.25 ml. or less may cause death in less than 24 hours, but the muscle lesions are not always quite as typical and are more moist with considerable spreading, gaseous, blood-tinged edema. Occasionally the liver may be light colored and exhibit minute small gray gaseous necrotic pockets 0.5 mm. or less in area. A generalized bacteremia occurs and the organism can be recovered from the lesion fluid, heart blood, and liver. Other laboratory animals are only slightly susceptible.

*Cl. septicum* has a wide range of susceptible animals. Guinea pigs and mice are very susceptible to small doses of 0.15 ml. to 0.2 ml. of 16 to 24 hour cultures or 0.5 ml. of lesion fluid. A spreading edema appears over the entire belly wall and thorax which may extend into the muscles. The coloration may vary from pink to dark red. It may be slightly gaseous. Different strains may vary in these features. The formation of long filaments of bacilli on the liver surface is a diagnostic feature for the presence of *Cl. septicum* in the injected animal, but it does not rule out the presence of other pathogens such as *Cl. perfringens* or *Cl. feseri* which do not form filaments or chains.

*Cl. novyi* is an important anaerobe in veterinary medicine. It is considered the lethal agent in black disease of sheep. Three toxin types, A, B, and C
have been recognized. From reports in the literature, types A and B are the ones commonly encountered.* The types occurring in animals need further investigation. This anaerobe has been recovered at least three times from liver lesions in cattle in Oregon. Antitoxin protection tests indicate that Type B *Cl. novyi* has been recovered from malignant edema-like infection in horses and has been found rather commonly in war wounds of man. *Cl. novyi* is not easy to recover and does not live well in culture. On suitable media a high level of toxin is produced in 24 to 48 hours. Marsh and Tunnicliff (8) found that a trypsinized liver medium produced the highest level of toxin.

Guinea pigs and mice are very susceptible to small doses of toxic cultures grown in cooked liver medium. Subcutaneous doses of 0.05 ml. or less into guinea pigs result in death in about 24 hours. A spreading gelatinous edema is present in the subcutaneous tissues. This may vary from light pink to dark red and drains out when the tissue is incised. The coloration may extend into the peritoneum. Lesions in the visceral organs are not usually of diagnostic significance.

The anaerobe can be recovered from the lesion fluid of guinea pigs, but not always from the heart blood as some old Pasteur Institute strains have killed animals with a sudden overwhelming toxemia without producing a bacteremia.

*Cl. hemolyticum*, the cause of bacillary hemoglobinuria, is one of the most fastidious of the Clostridia. It requires media with high tryptophane content and strict anaerobic conditions. Cooked liver or liver digest media provide most favorable nutritional requirements. It produces a hemolytic, necrotizing, and lecithinolytic toxin which causes a very rapid destruction of the erythrocytes of cattle, sheep, mice, and rabbits.

The highest level of toxin is produced in cooked liver or digest media usually reaching a peak in 16 to 18 hours. The pathogenicity for animals seems entirely dependent on the rapid hemolysis of erythrocytes and death results from anoxia. The intravenous injection of one to two mls. of a vigorous 16 to 18 hour culture into rabbits may cause death in four to six hours with hemoglobinuria and generalized hemoglobin staining of all tissues.

Subcutaneous or intramuscular inoculation of rabbits with 0.5 to 1.5 ml. of toxic culture results in death in 16 to 24 hours producing a hemoglobinuria, and a bloody transudate in the body cavities. Mice are readily killed in four to six hours with intravenous doses of as low as .02 ml. or less of toxic centrifuged supernatant fluid from toxic cultures or whole cultures. Guinea pigs weighing 300 to 500 gms. die in 16 to 36 hours with doses of 0.1 to 0.5 ml. of culture with a spreading nongaseous edema of the connective tissue and musculature. Hemoglobinuria and liver infarcts do not regularly occur in any of the inoculated animals. The anaerobe can be regularly recovered from the lesion fluid in cattle, but not with regularity from the

* Smith and Claus (7) reported that there is a correlation between toxin production and the digestion of casein or the blackening of iron-brain medium and that the less proteolytic group belong to *Cl. novyi* Type A, while the more proteolytic strains are of Type B.
heart blood. Intravenous injection of liver infarct saline suspensions does not produce death, but the organism may localize in the liver of rabbits and can be recovered in pure culture two or three days after injection.

*Clostridium sordellii* is closely related to *Clostridium novyi*, *Clostridium hemolyticum*, and *Clostridium bifermentans* in view of the similar cultural features. The slow proteolytic action in cooked meat media with the appearance of tyrosin-like crystals along with clotting and gradual digestion of the clot in litmus milk with liquefaction of coagulated serum, are distinct features not common to Type A *Clostridium novyi* and *Clostridium hemolyticum*. The colony features are often quite variable and may not be readily distinguished from the other three above mentioned anaerobes. Only a limited number of strains have been reported from human and animal sources. Three cultures recovered from cattle tissues retained their virulence and toxin-producing ability during 15 years of storage. A potent lethal toxin is produced. Rabbits and guinea pigs are killed in 16 to 18 hours following subcutaneous injection of 0.2 to 0.5 ml. of toxic cultures. A spreading gelatinous edema occurs around the site of injection which may vary from almost colorless to dark red according to the amount of the culture injected. The organism can be recovered from the blood and visceral organs. On the basis of toxin-antitoxin protection tests in laboratory animals, it is believed by some investigators to be a distinct species.

*Clostridium tetani* produces such a typical clinical syndrome in most animals that attempts to recover it from the affected animal are rarely done. The primary focus of infection is often difficult to locate, and when located, is usually very heavily seeded with a variety of other microbes. Recovery in cultures is a tedious job. Intestinal cultures are frequently found to contain this organism.

**ENTEROTOXEMIA**

*Clostridium perfringens* has been divided into six types, A, B, C, D, E, F, on the basis of the toxins produced. From these six types, 15 soluble antigenic exotoxins have been identified and are designated by letters of the Greek alphabet. Generally, one of the toxins is produced in quantity and is termed the major toxin, whereas other toxins are termed minor.

The toxins of *Clostridium perfringens* Type A, of which alpha is the major toxin, are responsible for the production of gas gangrene in man and occasionally in domestic animals. Type A enterotoxemia in sheep and cattle is characterized by ictero-hemoglobinuria. It also produces an enterotoxemia in suckling lambs.

*Clostridium perfringens* Type B produces two major toxins, beta and epsilon, and is responsible for lamb dysentery in England. It is a highly fatal disease occurring during the first few days of life. Type B has not definitely been isolated in the United States.

The major toxin of Type C is termed beta. It is a lethal and necrotizing toxin and identical to the beta toxin produced by Type B. In this country it causes an acute hemorrhagic enteritis in young calves and lambs. Sudden deaths in feedlot cattle have been attributed to Type C. Struck is caused by Type C and is an enterotoxemia-like disease of mature sheep in England.
"Cl. perfringens" Type D is the cause of enterotoxemia in sheep, goats, and cattle. It is generally distributed throughout the world. The primary toxic fraction of Type D is epsilon toxin which is a lethal and necrotizing toxin. Sudden deaths in feeder cattle have been attributed to Type D. Clinical signs of a CNS disturbance are valuable presumptive evidence of Type D intoxications.

For the purpose of this presentation it is impossible to deal with all of the toxic fractions of each type referred to in the literature. Three types, A, C, and D, have been identified in the United States. The major toxin fractions of these types are alpha, beta and epsilon. (Table I.) Each major toxin will be treated separately in the following discussion of typing. It is not the purpose of this paper to discuss in detail analytic methods but to present a rapid accurate means of gross identification. For example, analytic methods require the use of Swiss mice weighing 16 to 20 grams whereas gross identification of toxin permits the use of mice of practically any weight.

Two methods may be used to procure test material for toxin studies:

(1) Intestinal Contents: Specimens shipped to a lab should be sharp frozen and transmitted under good refrigeration, since destruction of toxin occurs soon after death. Sufficient toxin will be present in the intestine so that a direct test may be conducted. The concentration of toxin is most frequently found in the ileum. It is advisable to collect contents from other portions of the intestinal tract as well if there is evidence of inflammation and congestion. Myriads of organisms morphologically resembling "Cl. perfringens" may be found in smears of intestinal content. Wet mounts of intestinal contents, to which has been added a few drops of Lugols iodine solution, will reveal the presence of starch granules in Type D enterotoxemia of feeder lambs. These findings serve as a good means of presumptively diagnosing enterotoxemia. The contents of the intestine are "stripped out" into a container and diluted with an equal volume of distilled water or saline. The sample is centrifuged for approximately one-half hour at about 2,500 to 3,000 rpm. The supernatant is removed and used as the test material. No effort is made to sterilize the supernatant by filtration since the toxin, if present, will cause death prior to the possible effect of the bacteria present. If desired, antibiotics may be added to the supernate as a precautionary measure.

(2) Cultures: Two methods of preparing test material from cultures can be used: One consists of using the usual anaerobic plating technique and subculturing isolated colonies in a suitable media. The second technique consists of serially inoculating five or more tubes of suitable liquid media. The end of an inoculating needle is used to inoculate tube number one with a minute amount of material from the intestine and after the inoculum is dispersed, tube number two is inoculated from tube number one. This procedure is repeated until at least five tubes are inoculated. The media is incubated at 37°C for four to six hours at which time the highest dilution in which growth is apparent is used as inoculum for another series of five or more tubes. Usually the last tube in the second series will contain a pure culture of "Cl. perfringens." The supernatant obtained after centrifugation is
satisfactory for test material. This technique utilizes the rapid growth characteristic of *Clostridium perfringens* which virtually outgrow contaminating bacteria.

A suitable media that supports the production of the *Clostridium perfringens* toxins is Brewer's Media as modified by Vawter and Records and whose preparation can be found in the appendix. This media has been found to support the growth of all members of the genus Clostridium.

**Alpha Toxin**

Alpha toxin produces a hemoglobinuria in mice. Its positive identification can be made by means of the lecithovitellin reaction, which is described in the following protocol:

**Materials**
1. The usual serological equipment.
2. Egg solution prepared as follows—emulsify one yolk of a fresh egg in 200 cc. of CaCl₂—saline solution. Clarify to an opalescent solution through diatomaceous earth.
3. CaCl₂—Saline solution: To one liter of 0.9 percent NaCl solution add 2.75 cc. of a 1M CaCl₂ solution.
4. Standard antitoxin obtainable from the N.I.H.
5. Test material.

**Method**
1. Prepare serial dilutions of the test sample in 1 cc. quantities using CaCl₂ saline solution.
2. Add egg solution —1 cc.
3. Incubate three hours at 45° C.
4. Incubate at 5° C. overnight.
5. Read results to 50 percent end point.

**Interpretation**
Flocculation and the formation of a heavy layer of digested lecithin at the top of the tube indicates the presence of lecithinase and probably alpha toxin. The unit of measurement is Lf* /cc.

**Confirmation of alpha toxin and Lb determination:**
1. Set up dilutions of toxin to end point as before.
2. Set up two toxin controls using the highest dilution which previously gave a 4+ reaction and a 2+ reaction.
3. Add 0.5 unit of antitoxin contained in 1 cc. of CaCl₂ saline diluent to each tube.
4. Permit to neutralize for one hour at room temperature.
5. Add 1 cc. of egg solution to each tube.
6. Incubate three hours at 45° C. and overnight at 5° C.
7. Read results.

**Interpretation**
The neutralization of the lecithovitellin reaction in the dilution which previously reacted may be taken as positive identification.

*Lf*: The smallest quantity of toxin that results in 50 percent flocculation and digestion of lecithin in egg solution as prepared in the protocol.
of alpha toxin. If neutralization does not take place in the lower dilutions, the Lb of the toxin may be calculated as well. The highest dilution in which there is a partial reaction is computed to be 0.5 Lb. Roughly one Lb is equivalent to 50 to 100 Lf.

**Beta Toxin**

The mouse lethal test has been the test method of preference in many laboratories. It has been used to type cultures and toxic intestinal contents.

Materials: 1. Swiss mice (other strains may be used if Swiss mice are not available).
2. Specific Type C antitoxin. Do not use commercially available antitoxin unless assured by the producer that it is type specific and does not contain epsilon antitoxin.
3. The “unknown” clarified by filtration or centrifugation.

Procedure: **I—Toxin Determination**

1. All injections are made intravenously in either lateral tail vein. Difficulty has been encountered by some investigators in developing this technique. A one cc. tuberculin syringe equipped with 26 gauge needle, and an appropriate mouse holder are the basic equipment required. The primary prerequisite of a successful injection is to see that the mice are warm. At a temperature of 90-100° F. the tail veins of mice become dilated and are easily injected with the unknown. A goose-necked lamp with a reflector placed directly over the mouse cage will keep the animals sufficiently warm.

2. The unknown is injected in quantities rarely to exceed 0.2 cc. per mouse; never more than 0.4 cc. Two or three mice constitute a test group. All mice which suffer shock as evidenced by an immediate reaction are discarded from the test. Beta toxin will make itself evident in the animal in relationship to its concentration. One L.D. will usually cause illness in one or two hours and death in approximately eight hours. Ten or more L.D.’s will cause illness in 30 seconds to a minute and death in 15 to 30 minutes. The toxin concentration of Type C cultures varies widely as does that of intestinal contents.

Interpretation: Death of mice indicates that presence of toxin, the titer of which may be determined if so desired. Cultures may be expected to contain from 100 to 1,000 L.D., intestinal contents from one to 200 L.D.’s per cc.

**II—Toxin Neutralization**

1. Clostridium Perfringens Type C Antitoxin (Burroughs Wellcome & Co., Box 307, Tuckahoe 7, N. Y.) is diluted to contain approximately 10 antitoxin units per cc. Such dilution is necessary to eliminate the shock induced by the preservative (phenol) in the serum. One unit of antitoxin will neutralize approximately 20 L.D.’s of toxin.

2. Mix equal quantities of toxin and antitoxin in a common container and permit to neutralize for one hour at room temperature.
3. Inject mice as before using twice the volume found to be toxic previously, one-half of which is antitoxin. Also, inject toxin control mice as before. The L+ of the toxin may be determined if desired. It is based upon the smallest quantity of toxin which, when mixed with one unit of antitoxin and permitted to neutralize for one hour at room temperature, will cause death of 80 percent to 100 percent of the mice (16-20 gm. wt.) injected with the mixture.

Interpretation: The neutralization of the toxin identified it to be beta.

**Epsilon Toxin**

Methods of identification have been restricted to the use of the mouse toxicity test.

Cultures of Cl. perfringens Type B and Type D produce true epsilon toxin and prototoxin. This latter substance may be regarded as the precursor of the true toxin. It does not assume toxic characteristics until activated with trypsin or some other proteolytic enzymes. Activation may be accomplished by adding an equal volume of 0.5 percent trypsin solution (Difco 1:250) to cultures and incubating the mixture for 45 minutes at 37°C. The ratio of toxin to prototoxin concentration increases upon continued cultivation on artificial media. Many strains have a ratio of 1-100 or more.

Epsilon toxin in the digestive tract is completely activated and therefore requires no trypsin treatment.

**Identification**

Materials: The same as those described for beta toxin except specific Type D antitoxin is needed. Do not use commercial antitoxin unless assured by the producer that it is type specific and free from beta antitoxin.

Procedure: 1—Toxin Determination

The same procedure as described for beta toxin except that when testing culture toxin, it is necessary to activate prior to testing.

II—Toxin Neutralization

Interpretation: The presence of toxin which is neutralized by specific antitoxin identifies the toxin as epsilon.

**DISCUSSION**

In the event neither specific beta nor epsilon antitoxin neutralize the toxin in question, it is advisable to combine both epsilon and beta antitoxin and conduct a neutralization test. If neutralized, such a finding does not justify the conclusion that Type B is present. Individual colony isolates must be tested to eliminate the possibility that a mixed culture of Type C and Type D organisms exists.
TABLE 1
Tabulation of the Toxic Antigens of Cl. Perfringens Filtrates

<table>
<thead>
<tr>
<th>Name</th>
<th>Biological Activity</th>
<th>Biochemical Activity</th>
<th>Occurrence in Filtrates of Cl. Perfringens Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>Lethal, necrotic hemolytic</td>
<td>Lecithinase C</td>
<td>++++ + + + + +</td>
</tr>
<tr>
<td>Beta</td>
<td>Lethal, necrotic</td>
<td>?</td>
<td>− − − − − ++ − − − − ++</td>
</tr>
<tr>
<td>Gamma</td>
<td>Lethal</td>
<td>?</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Delta</td>
<td>Lethal, hemolytic</td>
<td>?</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Epilson</td>
<td>Lethal, necrotic</td>
<td>?</td>
<td>+ + ++ ++</td>
</tr>
<tr>
<td>Eta</td>
<td>Lethal—in doubt</td>
<td>? (±)</td>
<td>++ + ++</td>
</tr>
<tr>
<td>Theta</td>
<td>Lethal, hemolytic</td>
<td>?</td>
<td>++ + ++ ++</td>
</tr>
<tr>
<td>Iota</td>
<td>Lethal, necrotic</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Kappa</td>
<td>Lethal, necrotic</td>
<td>Collagenase ?</td>
<td>++ + ± +</td>
</tr>
<tr>
<td>Lambda</td>
<td>?</td>
<td>Proteolytic enzyme</td>
<td>+ + +</td>
</tr>
<tr>
<td>Mu</td>
<td>Spreading factor</td>
<td>Hyaluronidase</td>
<td>++ +</td>
</tr>
<tr>
<td>Mu</td>
<td>Affects capacity</td>
<td>Deoxyribonuclease</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td></td>
<td>of Leukocytes to stain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

± Produced by some strains.  + & + + Minor toxin.  ++++ Major toxin.

In order to complete the notes two anaerobic forms should be mentioned, both of which are encountered with some regularity. *Spherophorus necrophorus*, which is a strict anaerobe, but which does not form spores, can be cultured on the ordinary thioglycollate media with slight modification in procedure. The principal changes that are made are two in number. Since these organisms do not form spores, the contaminates cannot be killed by heating. In order to obtain a pure culture, the tissues must be cultured as soon after death as possible and extreme care must be used to avoid contamination in removing tissue for culture. Secondly, it is difficult to streak material suspected of containing Spherophorus because the contact with the air before anaerobic conditions can be produced may damage the organisms so they will not grow. For that reason, if it is necessary to pick a colony in order to obtain a pure culture, it is best to use a dilute inoculum in semi-solid agar in a pour plate or Burri tube.

This organism produces acid and gas from dextrose, lactose, sucrose, maltose, and salicin. Techniques used and explained previously for anaerobic bacteria apply here.

The second organism to be mentioned is *Actinomyces bovis*. This organism is one of several actinomycetaceae which are anaerobic. Aerobic ones are called Nocardia. Some claim two species of this one organism based mainly on whether the colony morphology is rough or smooth. Generally, the smooth form is isolated from lesions in animals. Pus is usually the material presented to the laboratory for making an examination, smears of which may show typical "sulphur granules." These are about one-half to three mm. in diameter and have irregular radiating structures quite characteristic of the species.
Transfer a typical granule to a clean glass slide, add a drop of two percent NaOH and crush under a coverslip. The typical radiating filaments are seen. After this inspection a Gram stain is applied on the same slide and the Gram-positive hyphe or fragments of them, observed. At the outer ends are club-shaped structures that do not take the Gram stain.

One must be cautious that similar appearing structures observed are not *Actinobacillus lignieri*. These, however, are Gram-negative and should not be confused. The absence of Gram's positive forms in lesion pus smears usually rules out the presence of *Actinomyces bovis*.

If the history and lesion is typical for *A. bovis* infection, culture need only be done to be sure that the organism in question does not liquefy gelatin or peptonize milk. If these are true, similar non-pathogens are ruled out.

In dealing with material for anaerobic culture, one should always be careful to examine aerobic spore formers in order to rule out the presence of *B. anthracis*. If in doubt, aerobic Gram-positive spore formers should be called to the attention of a qualified individual who is familiar with this species.

**CULTURE MEDIUMS FOR ISOLATION AND IDENTIFICATION**

All the medium described are adjusted with the aid of a glass electrode to pH 7.6 before autoclaving, with the exception of milk, which was adjusted to pH 6.8. All mediums are dispensed into five-eighth by six inch (1.5 by 15 cm.) tubes, eight cc. per tube, and autoclaved at 15 pounds for 30 minutes.

1. **Milk**—One hundred grams of dry milk is mixed with 1,000 cc. of cold water, strained through gauze, adjusted to pH 6.8 (more alkaline milk darkens on autoclaving) and dispensed to tubes to which 0.05 to 0.1 Gm. of reduced iron (Merck's "reduced with hydrogen") had previously been added. In this medium five to 100 organisms provides a suitable inoculum. Stormy fermentation and digestion, when they occur, are generally marked in less than 24 hours.

2. **Sugar-free base for fermentations**—

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bactopeptone or proteose peptone</td>
<td>20 gm.</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 gm.</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>Agar</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 cc</td>
</tr>
</tbody>
</table>

For fermentation reactions, the sugar in one percent amounts is added to the sugar-free base before tubing and autoclaving. Bromthymol blue is a satisfactory indicator, but since it tends to be reduced during the growth of anaerobes, it is added at the end of the growth period and the reactions are read at once. Cultures in the sugar-free base or with a nonfermentable sugar present remain on the alkaline side of this indicator. Fermentable sugars, without exception, are sufficiently broken down in less than 24 hours to give a frank acid reaction. Production of gas has not been considered, as some species produce it from peptones.
REPORT OF THE COMMITTEE

The most significant differences between the 20 odd species of gas gangrene bacilli are to be seen in their action on dextrose, lactose, maltose, salicin and sucrose.

(3) Liquefaction of Gelatin—

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>50 gm.</td>
</tr>
<tr>
<td>Bactopeptone</td>
<td>10 gm.</td>
</tr>
<tr>
<td>Sodium phosphate (Na₂HPO₄)</td>
<td>2 gm.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Sodium thyoglycollate</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 cc</td>
</tr>
</tbody>
</table>

All species in the group grow rapidly in this medium. Those which liquefy gelatin within 10 days give an extensive or a complete reaction within 24 hours. This medium without peptone gives good results, but a few species, especially Clostridium tetani, grow sparingly.

(4) Production of Hydrogen Sulfide—Most, if not all, Clostridium species produce at least a trace of hydrogen sulfide if a suitable substrate is supplied and a sufficiently sensitive indicator is used. Some laboratories do not routinely test for hydrogen sulfide.

(5) Formation of Indole—The sugar-free base (described under mediums for sugar fermentation) serves satisfactorily as a test medium for indole formation, but the following mixture gives more consistent results:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto tryptone</td>
<td>20 gm.</td>
</tr>
<tr>
<td>Sodium phosphate (Na₂HPO₄)</td>
<td>2 gm.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Agar</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 cc</td>
</tr>
</tbody>
</table>

All indole formers in the group give strongly positive reactions in 24 hours with Ehrlich’s reagent.

(6) Reduction of Nitrate— Cultures at 24 hours in the following medium give clear-cut reactions for nitrate with sulfanilic acid, dimethyla-naphthylamine reagent:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto tryptone</td>
<td>20 gm.</td>
</tr>
<tr>
<td>Sodium phosphate (Na₂HPO₄)</td>
<td>2 gm.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Agar</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>1 gm.</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 cc</td>
</tr>
</tbody>
</table>

(7) Mediums for Plating—Two procedures have been successfully followed in making isolations and in the study of colonial form: (a) ordinary surface plating on a firm agar and (b) subsurface growth in plates of semisolid medium.
LAB. NOTES ON ANAEROBIC BACTERIAL DISEASES

For surface colonies there are certain advantages in using a blood agar medium particularly as hemolysis or its absence provides useful information, while at the same time all the species grow luxuriantly. Good results follow the use of an infusion agar and three percent whole blood. An equal amount of blood added to one of the following clear plating mediums is equally satisfactory. Plates should not be over 24 hours old for Cl. Hemolyticum. streaked plates should be placed in anaerobic jar within 15 to 20 minutes.

For a clear plating medium, excellent results have been obtained with either of two formulas. The first is as follows:
Brewer's sugar-free broth with:

\[
\begin{align*}
\text{Dextrose} & \quad 0.1 \text{ percent} \\
\text{Agar} & \quad 2 \text{ percent} \\
\text{Adjust to pH 7.6.}
\end{align*}
\]

The second formula is as follows:

\[
\begin{align*}
\text{Proteose peptone} & \quad 20 \text{ gm.} \\
\text{Sodium phosphate (Na}_2\text{HPO}_4\text{)} & \quad 2 \text{ gm.} \\
\text{Dextrose} & \quad 1 \text{ gm.} \\
\text{Sodium thioglycollate} & \quad 1 \text{ gm.} \\
\text{Agar} & \quad 20 \text{ gm.} \\
\text{Water} & \quad 1,000 \text{ cc.} \\
\text{Adjust to pH 7.6.}
\end{align*}
\]

For subsurface colonies, the best results have been obtained with either of these clear plating mediums made with 0.75 percent of agar instead of two percent. For this purpose the agar must be perfectly clear. Satisfactory results have been obtained by boiling the medium, after adjustment to pH 7.6 for about five minutes and filtering it through paper under reduced pressure. The clear soft agar is autoclaved in 20 cc. amounts in tubes. When used it is melted, held in a 45 C. bath and inoculated with a highly diluted inoculum—enough to yield not more than 50 to 100 colonies per plate. Inoculated medium is poured into plates and incubated upright in an anaerobe jar or Brewer anaerobic petri dish lid.

ANAEROBIC FERMENTATION MEDIUM

\[
\begin{align*}
\text{Bacto tryptone} & \quad 20 \text{ gm.} \\
\text{Disodium phosphate (Na}_2\text{HPO}_4\text{)} \text{ CP} & \quad 1 \text{ gm.} \\
\text{Monobasic potassium phosphate CP} & \quad 0.1 \text{ gm.} \\
\text{Sodium chloride} & \quad 0.5 \text{ gm.} \\
\text{Agar} & \quad 3.5 \text{ gm.} \\
\text{Sodium thioglycollate} & \quad 0.1 \text{ gm.} \\
\text{Distilled water to make} & \quad 1,000 \text{ cc.}
\end{align*}
\]

Adjust pH to 7.6-7.7. Add phenol red stock solution (5 cc.), giving phenol red content of .02 gm. per liter.
**Biochemical Reactions of Clostridia**

<table>
<thead>
<tr>
<th>Species</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl. perfringens (welchii)</td>
<td>Stormy</td>
</tr>
<tr>
<td>Cl. butyricum (group)</td>
<td>Stormy</td>
</tr>
<tr>
<td>Cl. multifermentans</td>
<td>Stormy</td>
</tr>
<tr>
<td>Cl. aerofoetidum</td>
<td>Stormy</td>
</tr>
<tr>
<td>Cl. tertium</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. fallax</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. paraputrefteum</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. carnies</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. chauroei (feseri)</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. septicm</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. septicm</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. spbenoides</td>
<td>Acid</td>
</tr>
<tr>
<td>Cl. novyi (oedematiens)</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. bifemerntans</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. sordeleii</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. sporogenes</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. histolyticum</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. tetanomorphic</td>
<td>No change</td>
</tr>
<tr>
<td>Cl. difficile</td>
<td>No change</td>
</tr>
<tr>
<td>Cl. capitoriavis</td>
<td>No change</td>
</tr>
<tr>
<td>Cl. chichiarium</td>
<td>No change</td>
</tr>
<tr>
<td>Cl. tetani</td>
<td>No change</td>
</tr>
<tr>
<td>Cl. botulinum</td>
<td>Digested</td>
</tr>
<tr>
<td>Cl. hemolyticum</td>
<td>No change</td>
</tr>
</tbody>
</table>

Distribute 9 ml. of basal medium in 15 x 150 mm. capped tubes. Sterilize 15 minutes at 121°C in wire racks. Carbohydrates and other fermentable substances can be added to aliquot parts of the basal medium prior to sterilization to make one percent final concentration. Some rare sugars and maltose may hydrolyze during pressure sterilization. These can be filtered and subsequently added to the basal media aseptically to make one percent concentration. Another method is to sterilize carbohydrates separately in 10 percent solution in distilled water for 10 minutes at 10 lbs. pressure and add 1 cc. of the carbohydrates or other fermentable substances to aliquot parts of the basal medium aseptically. This will require melting of the basal medium and rotating each tube after adding the sugars to assure proper distribution in each tube. Dextrose, fructose, lactose, sucrose, salicin and glycerol are the ones most commonly used for pathogenic sporulating anaerobes. Salicin solubility is only about 0.3 percent in distilled water. Arabinose, xylose, inulin, soluble starch, and others are occasionally used. Glycerol is usually used in five percent concentration. C.P. glycerol is likely to be quite acid and should be neutralized to about pH 7.5 with M/15 disodium phosphate. It changes pH slightly on pressure sterilization. This
formula has been used with Cl. chauvoei, Cl. septicum, Cl. sporogenes, Cl. novyi, Cl. sordellii and Cl. hemolyticum with satisfactory results. Boil for five minutes before inoculation. Inoculate into deeper stratum with sterile capillary pipettes.

**Phenol Red Stock Solution (Clark and Lubs formula)**

Dissolve 0.1 gm. phenol red phenolsulphthalein in 5.7 N/20 NaOH in a glass mortar, then dilute to 25 cc. with distilled water. Solution keeps well in stopped bottle.

**COOKED LIVER MEDIUM FOR SPORULATING ANAEROBES**

Modified by L. R. Vawter, Oregon State College

Fresh bovine liver obtained direct from a slaughter plant is desirable. Half or whole liver can be chilled overnight, then cut up into 1 or 2 lb. pieces, wrapped, and placed in freezer to be used as needed. It will usually keep well for about two to three months.

Liver infusion broth is prepared by infusing 500 gms. of the ground beef liver in 1,000 ml. of distilled water with 0.5 percent NaCl several hours or overnight in cold room. Cook slowly for about 40 minutes with occasional stirring to break up clumps until broth is clear. Strain off broth and save the cooked liver which should be pressed dry, reground, and rubbed to fairly fine particles.

Place about 1 cm. of ground liver in 20 x 150 culture tubes with caps. Add about 200 mg. of CaCo₃ to each tube. Add a similar amount of iron filings or a 5 cm. length of iron wire to each tube.

Make up volume of the liver broth to 1 liter then add:

- **Proteose peptone** ................................. 10 gm.
- **Dextrose** ............................................. 1 gm.
- **Dibasic potassium phosphate** ............... 2 gm.
- **Sodium thioglycollate** ......................... 0.1 gm.

Adjust pH to 7.8-8.0.

Heat and filter off sediment.

Add sufficient liver broth to the cooked liver tubes to a final depth of 7-8 cm. The final pH after sterilization should be 7.0-7.4.

Sterilize 20 minutes at 15 lbs. pressure.

Final pH of broth should range from 7 to 7.4 and is influenced by acidity of the liver used for the medium. Butcher shop liver is likely to be quite acid and not always satisfactory. The finished medium can be stored at room temperature, or in the refrigerator.

Even though most sporulating anaerobes will start growth promptly, boiling in a water bath just before use is advisable. Dextrose can be omitted for stock cultures. Increasing the dextrose to 0.2 percent seems advantageous for Cl. feseri. This medium has proved very satisfactory and gives excellent growth with the clostridial organisms; i.e., Cl. feseri, Cl. perfringens, Cl.
septicum, Cl. novyi, Cl. hemolyticum, Cl. sporogenes, and others such as Actinomyces necrophorus and many aerobes. Liver infusion agar for plates or deep agar shake cultures can be prepared with the basal fluid by omitting the cooked liver and CaCO₃. (pH of agar 7.2-7.4.)

**Anaerobic Indicator Solution**

Prepare—
1. 6.0 ml. N/10 NaOH diluted to 100 ml. with distilled H₂O.
2. 3.0 ml. 0.5 percent aqueous methylene blue diluted to 100 ml. with distilled H₂O.
3. 6.0 gms. dextrose 100 ml. distilled H₂O + a small crystal of thymol.

Use: Mix equal parts of the three solutions and boil until decolorized. Place tube in jar and begin air evacuation. In systems where carbon monoxide is present, this system will not work.

**REFERENCES**

10. Willis, A. T.: A Rapid Method for Purification of Some Clostridia from Mixtures with Other Organisms Especially the Aerobic Spore Formers.
CONSTITUTION AND BY-LAWS
OF THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME

The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE

The purpose of this Association shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of the laws, regulations, policies and methods pertaining to milk and meat hygiene, and to the prevention, control and eradication of transmissible livestock diseases; to maintain co-ordination among the various livestock regulatory organizations, and to serve as livestock sanitary science clearing house between this Association and the following: The livestock owner, the livestock sanitarian, the milk and meat hygienist, the veterinary practitioner, the transportation and stock yard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "livestock" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP

There shall be three kinds of members—Official and Individual and Non-Voting Junior.

OFFICIAL MEMBERSHIP

The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, Puerto Rico, the Virgin Islands and Los Angeles County, California shall be eligible to official membership in this Association and be represented on the Executive Committee by the livestock sanitary executive official.

INDIVIDUAL MEMBERSHIP

Any person engaged in livestock sanitary work for Federal, provincial, state, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.
CONSTITUTION AND BY-LAWS

JUNIOR NON-VOTING MEMBERSHIP

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

ARTICLE IV—MEETINGS

The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS

The officers of this Association shall be: President, First Vice-President, Second Vice-President, Third Vice-President, Secretary-Treasurer, and an Executive Committee.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various States, the Director of Livestock Regulatory Programs of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive regulatory officer of Cuba, Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, the elective officers of this Association and eight delegates at large representing the livestock industry including poultry.

No more than two delegates from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast; consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; the North central, consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; the Southern, comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the Western district consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry, including poultry. No more than two (2) delegates at large shall be elected from each of the four designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.
73 The Executive Committee shall constitute the administrative body of
74 this Association and shall determine its activities and policies.
75 All recommendations and reports of officers and committees shall be
76 referred for consideration to the Executive Committee.
77 The First Vice-President shall be ex-officio chairman of the Executive
78 Committee.
79 The Executive Committee shall elect yearly a Secretary-Treasurer for
80 the Association. The Secretary-Treasurer shall receive such salary and
81 allowance as may be fixed by the Executive Committee.
82 The Executive Committee shall cause to be audited annually or oftener
83 if deemed necessary, the receipts and disbursments of the Secretary-
84 Treasurer, and shall have authority to hear and determine all complaints
85 filed before it in writing relative to the conduct of any member; and
86 shall have authority to accept or reject applications for individual mem-
87 bership properly placed before them. Three negative votes shall dis-
88 qualify for such membership.

ARTICLE VI—PROGRAM COMMITTEE

90 The President, the Chairman of the Executive Committee and the Sec-
91 retary-Treasurer and the Chairman of the respective committees shall
92 constitute the Program Committee. It shall be the duty of the officers of
93 the Program Committee to make the necessary arrangements and provide
94 the program for the annual and special meetings.

ARTICLE VII—DUTIES OF OFFICERS

96 1. President: It shall be the duty of the president to preside at all
97 meetings of this Association; to appoint all committees excepting the
98 Executive and Officer faction of the Program Committee; to call
99 special meetings of the Association whenever he considers the holding of
100 such meetings necessary for the good of the livestock industry or upon
101 the written request of five members of the Executive Committee. The
102 president shall be an ex-officio member of all committees.
103 2. First Vice-President: The first vice-president shall be chairman of
104 the Executive Committee. In the absence of the president, he shall
105 preside at the meetings of the Association. In the event of the absence,
106 disability or resignation of the president he shall perform all duties of
107 the president. He shall be an ex-officio member of the Executive and
108 Program Committees.
109 3. Second Vice-President: The second vice-president shall assume the
110 duties of the president in the event of the absence, disability or resigna-
111 tion of the president and first vice-president. He shall assume the chair-
112 manship of the Executive Committee in the event of the absence, dis-
113 ability or resignation of the first vice-president. He shall be an ex-officio
114 member of the Executive Committee.
115 4. Third Vice-President: The third vice-president shall assume the
116 duties of the president in the event of the absence, disability or resigna-
117 tion of the president, first vice-president and second vice-president. He
118 shall assume the chairmanship of the Executive Committee in the event
119 of the absence, disability or resignation of the first and second vice-
120 presidents. He shall be an ex-officio member of the Executive Committee.
121 5. Secretary-Treasurer. The Secretary-Treasurer shall keep an ac-
122 curate record of the proceedings of the Association. Whenever au-
123 thorized so to do by the Executive Committee he shall publish said
124 proceedings and distribute them to the members of the Association. The
125 Secretary-Treasurer shall also keep an accurate record of the proceedings
126 of the Executive Committee and shall furnish a copy to each member of
127 said Executive Committee. He shall forward to each Executive Com-
128 mittee member a copy of each regulation approved by the Association.
129 He shall keep an accurate account of all Association moneys received
130 and disbursed. He shall also present to the Chairman of the Executive
131 Committee a list giving the name, occupation and address of each ap-
132 plicant for individual membership for the approval of the Executive
133 Committee. He shall perform such other duties as may be authorized
134 and prescribed by the Executive Committee. He shall be ex-officio sec-
135 retary of the Executive Committee, also an ex-officio member and
136 secretary of the Program Committee. He shall be bonded for not less
137 than ten thousand dollars.

138 ARTICLE VIII—AMENDMENTS
139 The constitution of this Association may be amended by a two-thirds
140 vote of the members of the Association present and voting at an annual
141 meeting, provided that the specific amendment to be acted upon shall
142 have been presented in writing at a previous annual meeting and further
143 provided that the amendment has received the approval of the Executive
144 Committee.

145 BY-LAWS
146 ARTICLE I—ORDER OF BUSINESS
147 Registration.
148 Call to Order.
149 Report of Secretary-Treasurer.
150 President’s Address.
151 Reading of Papers.
152 Committee Reports.
153 Discussion.
154 Unfinished Business.
155 New Business.
156 Nomination and Election of Officers and eight members to Executive
157 Committee.
158 Adjournment.
159 A suspension of the By-laws may be made by a two-thirds majority
160 for the purpose of changing the order of business or to facilitate im-
161 portant business.
ARTICLE II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary-Treasurer. The Application shall give the name, occupation and address of the applicant and shall be accompanied by a fee of five dollars ($5.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary-Treasurer, who shall in turn submit them to the Executive Committee. An individual member may be expelled for cause by the Executive Committee.

ARTICLE III—MEETINGS

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association. The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV—QUORUM

Twenty-five members of the Association shall constitute a quorum. Twenty members of the Executive Committee shall constitute a quorum.

ARTICLE V—DUES

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association. The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association. The dues for official memberships shall be 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
J. Milligan

Individual members
J. Armstrong
E. T. Braye
G. W. Cooper
J. E. Greene
M. K. Heath
G. D. Ingram
A. B. Larsen
B. N. Lauderdale
J. Milligan
C. H. Poitevint
C. S. Roberts

ALASKA

Official member
J. Honsinger

Individual members

ARIZONA

Official member
W. M. Thompson

Individual members
Circle One Livestock Co.
L. H. Fuller
John Jacobs Farms
E. R. Mackery

ARKANSAS

Official member
D. Ibsen

Individual members
O. E. Harrison
E. H. Peterson

CALIFORNIA

Official member
J. E. Stuart

Individual members
D. Addis
M. J. Algee
R. A. Bankowski
L. E. Bartelt
R. S. Bishop
J. C. Boer
R. C. Boobar
A. G. Boyd
Brennan & Laskey
D. L. Bristow
V. C. Bunker
Cal. Auction Yard Ass'n
California Cattlemens' Ass'n
Cal. Farm Bureau
Calif. Veterinary Med. Association
J. Carricaburu
N. H. Casselberry
J. Chapman
E. F. Chastain
J. F. Christensen
C. J. Claire
D. S. Clark
N. M. Clinton
J. C. Davidson
H. N. Davis
J. M. Davison
G. L. Dayman
C. A. Delli Quadri
R. S. Dickson
M. K. Dunlap
A. C. Emminger
J B. Enright
T. B. Evilie
C. J. Ferreira
R. W. Fogleman
J. N. Fulmer
O. A. Ghiggiole
C. L. Gooding
M. S. Gordon
J. A. Gourlay
T. J. Hage
R. H. Haight
P. Haims
J. Hart
W. E. Hawkins
B. R. Heron
W. P. Heuschele
R. J. H. Holte
R. H. Huffaker
W. P. Humphrey
L. M. Hurt
H. C. Jackson
MEMBERSHIP ROSTER

T. W. Jackson
S. L. Jamison
D. E. Jasper
H. N. Johnson
K. L. Johnson
D. W. Jolly
E. E. Jones
F. P. Jones
S. V. Jones
H. B. Keith
A. Kelly
J. W. Kendrick
J. King
C. H. Kinsley
G. K. L. Knott
L. H. Krum
C. A. Lamb
J. D. Lamont
L. R. Libby
D. L. Mace
S. H. Madin
R. C. Maris
W. Matlch
R. W. McFarland
B. McGowan
R. W. McIntyre
K. G. McKay
D. G. McKercher
R. W. Merriman
K. F. Meyer
L. D. Meyers
J. Nehay
W. Ofenheim
C. H. Ozanian
J. C. Pace
R. H. Packard
H. H. Page
R. C. Park
J. B. Parr
H. H. Parrell
F. L. Pellessier
F. B. Pulling
E. R. Quortrup
G. A. Railsback
R. D. Richards
C. E. Robinson
W. H. Rockey
A., S. Rosenwald
W. L. Rottman
W. W. Sadler
F. F. Saint
San Diego County

F. H. Saunders
K. Schaaf
O. W. Schalm
R. J. Schermerhorn
H. J. Schmidt
R. C. Schock
C. R. Schroeder
F. S. Scott
L. J. Scott
K. C. Seeger
C. K. Shane
D. D. Sharp
E. F. Sheffield
G. Schultz
O. W. Sommer
J. L. Sorenson
A. T. Spencer
M. A. Stedham
D. E. Stover
D. E. Suther
A. L. Tietze
O. H. Timm
J. Traum
G. N. Tucker
Tulare Veterinary Hospital
W. D. Urban
H. Van Dam
G. K. Van Vleck
J. D. Vietti
W. J. Ward
W. W. Watkins
J. S. Watson Dairy
G. B. E. West
R. W. Wichmann
W. P. Wing
G. Wise
H. G. Wixom
W. W. Worcester

LOS ANGELES COUNTY

Official member
R. J. Schroeder

Individual members
P. A. Asplund
W. H. House
R. C. Hubbard
R. H. Hurt
V. M. Makoff
M. E. McElroy
L. F. Meier
M. D. Moys
J. Pecaro
MEMBERSHIP ROSTER

R. L. Phillips
R. Robusto
R. J. Schroeder
W. A. Young

COLORADO

Official member
W. C. Tobin
Individual members
O. R. Adams
W. W. Brown
T. L. Chow
W. A. Clark, Jr.
E. J. Cole
J. R. Collier
C. L. Davis
J. C. Flint
N. Frank
R. M. Gow
A. B. Hoerlein
M. Huff
W. E. Johnson
W. O. Kester
B. R. McCrory
J. S. Osborn, Jr.
H. E. Schaulis
B. Shambaugh
W. C. Tobin

CONNECTICUT

Official member
J. V. Smith
Individual members
J. W. Beck
F. Ferrigno
J. Hwang
E. Jungherr
B. Lipman
J. V. Smith

DELTA OF COLUMBIA

Official member
C. D. VanHouweling
Individual members
A. Z. Baker
F. L. Herchenroeder
J. Hourrigan
O. J. Hummon
N. Konnerup
J. J. Martin
J. A. McCallam
N. L. Meyer
A. R. Miller
C. H. Pals
B. C. Pier
A. F. Ranney
E. E. Saulmon
H. W. Schoening
L. A. Spindler
J. Splitter
A. L. Tellejohn
H. F. Wilkins

FLORIDA

Official member
C. L. Campbell
Individual members
R. H. Bennett
D. E. Cooperrider
I. P. Coulter
E. M. Ellis
J. G. Fish
J. G. Fish, Jr.
C. R. Forman
J. H. Gainer
J. H. Graves
W. F. Jackson
V. C. Johnson
D. L. Lichty
J. E. B. Mouw
W. Powell
W. R. Pritchard
C. Reid
J. E. Scatterday
W. L. Sippel
L. E. Swanson
C. Zillman
M. R. Zinober
| GEORGIA |
|------------------|------------------|
| Official member  | J. W. Mann       |
| Individual members |                |
|                  | O. M. Bateman    |
|                  | H. B. Hodgson    |
|                  | W. T. Hubbert    |
|                  | T. J. Jones      |
|                  | L. H. Karstad    |
|                  | R. J. Lee        |
|                  | J. Lieberman     |
|                  | C. J. Mikel      |
|                  | A. M. Mills      |
|                  | L. A. Mosher     |
|                  | J. Russell, Jr.  |
|                  | S. C. Schmittle  |
|                  | L. E. Starr      |
|                  | J. H. Steele     |
|                  | F. T. Sutton     |
|                  | J. M. Sutton     |
|                  | E. S. Tierkel    |

| HAWAII |
|------------------|------------------|
| Official member  | E. H. Willers    |
| Individual members |                |
|                  | G. H. Murphy     |
|                  | Kualoa Ranch     |
|                  | E. H. Willers    |

| IDAHO |
|------------------|------------------|
| Official member  | A. P. Schneider  |
| Individual members |                |
|                  | F. W. Frank      |
|                  | Idaho Sheep Commission |
|                  | H. L. McEwan     |
|                  | A. P. Schneider  |
|                  | J. W. Stucki     |

| ILLINOIS |
|------------------|------------------|
| Official member  | A. K. Merriman   |
| Individual members |                |
|                  | R. F. Baker      |
|                  | D. E. Bartlett   |
|                  | P. D. Beamer     |
|                  | C. E. Byler      |
|                  | W. J. Boddington |
|                  | L. E. Boley      |
|                  | A. C. Bolle      |
|                  | A. E. Bott       |

|                | A. R. Bott       |
|                | H. Caldwell      |
|                | C. L. Clark      |
|                | A. J. Coale      |
|                | J. W. Cunkelman  |
|                | H. C. Curtis     |
|                | L. R. Davenport  |
|                | P. L. De Puy     |
|                | L. A. Dykstra    |
|                | J. O. Gwin       |
|                | J. G. Hardenbergh|
|                | V. A. Haring     |
|                | M. J. Harvey     |
|                | J. R. Hay        |
|                | H. E. Held       |
|                | C. B. Hostetler   |
|                | Institute Amer. Poultry Ind. |
|                | W. E. Jennings   |
|                | R. C. Kamm       |
|                | E. C. Khuen      |
|                | C. H. Koonz      |
|                | B. L. Lake       |
|                | A. J. Legner     |
|                | N. D. Levine     |
|                | W. C. Logan      |
|                | F. C. Mau        |
|                | W. B. McCannon   |
|                | A. C. Merrick    |
|                | A. K. Merriman   |
|                | C. A. Metz       |
|                | Mid-West Order Buyers |
|                | A. G. Misener    |
|                | C. Mohme         |
|                | R. L. Morin      |
|                | C. H. Myers      |
|                | G. Novotny       |
|                | J. F. Palmer     |
|                | D. A. Price      |
|                | J. D. Ray        |
|                | H. C. Rinehart   |
|                | C. M. Rodgers    |
|                | M. D. Schneider  |
|                | W. Schwab        |
|                | O. W. Seher      |
|                | J. Simon         |
|                | H. L. Sparks & Co. |
|                | S. S. Swift      |
|                | W. L. Wake       |
|                | M. H. Watkins    |
|                | L. M. Webb       |
|                | E. J. Wilson     |
|                | H. E. Wilson     |
|                | B. E. Wise       |
## MEMBERSHIP ROSTER

### INDIANA

<table>
<thead>
<tr>
<th>Official member</th>
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<tbody>
<tr>
<td>J. W. Green</td>
<td>Arnold Laboratories</td>
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<tr>
<td>L. R. Barnes</td>
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<tr>
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<td>Conner Prairie Farm</td>
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<td>J. M. Droge</td>
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<td>W. D. Yoder</td>
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### IOWA

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<tr>
<td>A. L. Sundberg</td>
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<td>D. Garner</td>
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<td>W. A. Hagan</td>
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### KANSAS

<table>
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<tbody>
<tr>
<td>A. G. Pickett</td>
<td>Armour &amp; Co.</td>
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<td>R. R. Dykstra</td>
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<td>V. D. Foltz</td>
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<td>E. J. Frick</td>
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<td>N. D. Harwood</td>
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<td>R. W. Menges</td>
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<td>A. G. Pickett</td>
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<td></td>
<td>J. A. Porter</td>
</tr>
<tr>
<td></td>
<td>M. J. Twiehaus</td>
</tr>
</tbody>
</table>
MEMBERSHIP ROSTER

KENTUCKY
Official member
R. H. Singer
Individual members
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