PROCEEDINGS
SIXTY-SEVENTH
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK SANITARY ASSOCIATION
WESTERN SKIES HOTEL
Albuquerque, New Mexico
October 15-16-17, 1963
PROCEEDINGS
SIXTY-SEVENTH
ANNUAL MEETING

of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

WESTERN SKIES HOTEL
Albuquerque, New Mexico
October 15-16-17-18, 1963
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<td>1. Sept. 27–28, 1897**</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Forth Worth, Texas</td>
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<td>2. Oct. 11–12, 1898</td>
<td>Omaha, Nebraska</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kansas</td>
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<td>5. Oct. 8–9, 1901</td>
<td>Buffalo, New York</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
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<td>6. 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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<td>8. Aug. 23–24, 1904</td>
<td>St. Louis, Mo.</td>
<td>*Dr. J. C. Norton, Arizona</td>
<td>*Mr. Wm. P. Smith, Monticello, Illinois</td>
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<td>31. Nov. 30 – Dec. 1–2, 1927</td>
<td>Chicago, Ill.</td>
<td>*Dr. L. Van Es, Lincoln, Nebraska</td>
<td>*Mr. O. E. Dyson, Wichita, Kansas</td>
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<td>32. Dec. 5–6–7, 1928</td>
<td>Chicago, Ill.</td>
<td>*Dr. C. A. Cary, Auburn, Alabama</td>
<td>*Mr. O. E. Dyson, Wichita, Kansas</td>
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<td>34.</td>
<td>Dec. 3-4-5, 1930</td>
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<td>*Dr. A. E. Wight, Washington, D. C.</td>
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<td>36.</td>
<td>Nov. 30 - Dec. 1-2, 1932</td>
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<td>37.</td>
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<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
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<td>*Dr. H. D. Porter, Cheyenne, Wyoming</td>
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<td>47.</td>
<td>Dec. 1-2-3, 1943</td>
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<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
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<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
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<td>49.</td>
<td>Dec. 5-6-7, 1945</td>
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<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
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<td>50.</td>
<td>Dec. 4-5-6, 1946</td>
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<td>*Dr. William Moore, Raleigh, N. Carolina</td>
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<td>51.</td>
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<td>*Mr. Will J. Miller, Topeka, Kansas</td>
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<td>53.</td>
<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N. D.</td>
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<td>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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<td>58.</td>
<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W. Va.</td>
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<td>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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<td>60.</td>
<td>Nov. 28-29-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
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<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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<td>62.</td>
<td>Nov. 4-5-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Dr. John G. Milligan, Montgomery, Alabama</td>
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<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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<td>65.</td>
<td>Oct. 3-Nov. 1-2-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
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<tr>
<td>67.</td>
<td>Oct. 15-16-17-18, 1963</td>
<td>Albuquerque, N. M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R. I.</td>
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</table>

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

R. A. Hendershott
Trenton, New Jersey

We are thankful, O Lord, that so many of us have been permitted to be at this our Sixty-seventh Annual Meeting.

We are thankful that comparative peace has prevailed throughout the world and that the tensions that greeted the opening of the 66th Annual Meeting have lessened.

We ask thy divine guidance in all of our efforts; grant us, we beseech Thee, wisdom, understanding, integrity, energy, and an abiding desire for truth in our daily endeavors to unlock the facts of nature as they apply to the work in which we are engaged.

We ask Thy blessing on all that we do at this meeting so that our collective effort will be of benefit to the health and well-being of both man and animal.

We ask Thy blessing and guidance to all those charged with authority, to the end, that Thy will shall be done.

Amen

The President then called upon Dr. J. G. Milligan to introduce the next speaker.

Dr. J. G. Milligan: It is with a great deal of pride and humility that I accept this opportunity to introduce our next speaker. He happens to be Mister United States Livestock Sanitary Association. He attended his first meeting in 1905 and attended 57 consecutive meetings. No one else has ever come close to that record. Today he is going to extend his welcome to us for being here. It was through his efforts that we were privileged to come here. At this time I want to present to you Doctor F. L. Schneider. Applause.
ADDRESS OF WELCOME

F. L. Schneider

Albuquerque, New Mexico

In welcoming the United States Sanitary Livestock Association to its 67th Annual Meeting here in Albuquerque, I wish to say at the outset that I do so with a great deal of personal pleasure. I looked forward to this occasion for many years. It is regrettable that our good Senator Clinton P. Anderson and our Junior Senator E. L. Mechem could not be here. Being invited to pinch hit for them is an honor greatly appreciated.

However, not being accustomed to public speaking as I am, I feel that I have been placed somewhat in the position of a certain ancient man of old.

History records that in ancient times there lived two very important personages, Mark Anthony and Cleopatra by name. It is recorded that Mark and Cleopatra, as did the more prominent livestock people of that time, dwelled in tents on the open range. It is further recorded that one night, when the hour was late and the moon was low, Mark betook himself to the tent of Cleopatra and rapped on the tent pole that held the flap of her tent. Cleopatra, being a light sleeper and expecting Mark Anthony, went to the flap and opened it saying, "Why, Mark, what are you doing here at this hour of the night?"

Mark replied, "Well, Cleopatra, I assure you I did not come here to make a speech." Laughter.

I do not intend to make a speech or an address either and don't mistake me that I think I am capable of taking the place of either one of our illustrious Senators; however, as Henry Ford once said about his Ironton Railroad—"My railway isn't as long as the Pennsylvania but it is just as wide."

I don't propose to phrase this welcome in the flowery speech that others might. I do wish to assure you that it is equally sincere.

Now another little pun. It is said that a very famous minister of the later years of the last century, Henry Ward Beecher, once said to a young minister who asked him one Sunday morning, "Henry, about how long do you make your sermons?"

"Well, sir," he said, "I found that few souls are saved after about 20 minutes."

So Gentlemen, I am going to make this very brief. In the first place, if I make it longer than a few moments, I know I will, if I have not already done so, forget many of the nice things I fully intended to say to you.

Briefly, we wish to welcome you here from the bottom of our hearts and hope that we speak for you a very successful meeting. We want you to come back and visit us again. In fact, if you are sufficiently impressed by your present visit we hope you may come and abide with us permanently.
Now just a word about New Mexico. This state has made some very desirable progress during the last two decades. I think perhaps some of you have heard me say before that this is the land where the sunshine spends the winter. Something I think we may boast about is that here we have the four seasons but not in their intensity. Now what I think we need more than any other thing is somewhat of a population explosion from within and we need some more good folks from the outside to come and make their home here with us. And above everything else we need rain. That may sound odd to some of you folks but it is a fact.

To illustrate how serious that is, a few winters ago I had occasion to visit an old and prominent Indian in one of our Indian Pueblos. He was one of the early graduates of the first Indian School in the United States. He was a friend of mine and I knew him well. I said to him, "Wan, how are things going here?"

"Well, Doctor," he said, "They're bad; we need rain badly."
I said, "Yes, I know but it is going to rain."
"No, Doctor," he said, "I remember one time when it never did rain at all." Laughter.

I think we are keeping up with the times to some extent, but having passed into the ninth decade of my life, things are going very rapidly for me. In fact, small things are always happening which are surprising to me.

Albuquerque is a city for its size that ranks amongst the foremost in the United States as to expansion of area within the city limits and population. Less than 20 years ago Albuquerque had 30,000 residents and an area of seven or eight square miles, and today we may boast of almost a quarter of a million population and over 125 miles in area. I think that speaks for itself as far as this city is concerned.

I think that one reason for that is the climate; another reason is that Albuquerque and New Mexico have been very kind to all who came here.

Now I merely want to say a word as your host. We do want to do for you. If you find anything you desire that is not illegal or illegitimate, we will try and help you out. May I suggest that in that event you contact our most efficient secretary Ralph Hendershott who many of you know is the one man who can get the job done.

Now there is just one other remark. If any of you during the week find yourself short of what we call filthy lucre, won't you contact my good friends, Doctors V. H. Magatagon or L. E. Bodenweiser, and I am quite sure either one will be prompt to do something about it. Gentelmen, I thank you.
RESPONSE TO WELCOME

C. L. Campbell
Tallahassee, Florida

Doctor Schneider, Members of the Association:—Like Mark Anthony, by the time you leave here I am sure you will realize I did not come here to make a speech.

We are very pleased, Doctor Schneider, to meet here in the land of enchantment at our Sixty-seventh Annual Meeting. Actually this is the first time that this group has met in New Mexico. I believe, however, this is the third time that we have met in the southwest. The first time was in Fort Worth, Texas, in 1897—this was the first printed record of a meeting of this Association. The second time was on the occasion of our Fifty-fourth Annual Meeting in Phoenix, Arizona in 1950.

We do appreciate this invitation very sincerely.

I have been greatly impressed with what I have seen in this area, and while this state ranks 37th in population, having probably about a million persons, I understand it is fifth in area among our states. I understand it extends some 400 miles North and South, and 350 miles East and West.

While the history of New Mexico may date back hundreds of years, the state can truly be called the land of youth which associates its name with atomic bombs, missiles, and space flights; these are the terms that we associate with the youth of today. In the town of Los Alamos one of every thirty residents has a doctor of philosophy degree and averages about 40 years of age.

One of the things that has impressed me here in Albuquerque is the tremendous vision that one gets when he goes out of doors.

I had the opportunity Monday afternoon to go up on the Sandia Crest and while I did not know how much I was seeing, after I got down I understand that I had a panoramic view of some eleven thousand square miles; that is a tremendous view.

As far as Albuquerque itself is concerned I understand while it is 1,000 feet below the peak of Sandia Crest, it is still some 5,300 feet above sea level, which I understand makes it one of the highest metropolitan cities in the United States. This information I received from the Chamber of Commerce brochures: namely that the sun shone every day during 1962. This allows the city to lay claim to the Sunshine Capital of the United States. Being from Florida, the sunshine capital of the world, I shall not comment.

I am greatly impressed by the vastness of your state. I understand that one of your fine citizens at Roswell, Mr. Anderson, owns more land than any other man in the United States—1,135,000 acres. It is difficult for one to envision just how much land that is. If, however, we were to compare it to the size of the State of that of our President Doctor Grennan, we find that it is nearly twice the size of Rhode Island.
Here again, then, is a reference to vastness. While I consider Florida one of our frontier states with large spreads of land, if we consider Florida by comparison it is only half vast.

Doctor Schneider, it is a pleasure to accept your invitation to meet here with you today and on behalf of the United States Livestock Sanitary Association, we thank you.
PRESIDENT'S ADDRESS
T. J. Grennan, Jr.
Providence, Rhode Island

Ladies and Gentlemen, Distinguished Guests, Visitors, and Members
of the United States Livestock Sanitary Association: It is a pleasure to
add my welcome to each of you to that already extended. Those of us that
come from the early colonial states realize that we are relatively new-
comers upon arriving here in "The Land of the Missions," and "The Land
of Enchantment."

Many times in the past, the address presented to this body of my
predecessors made mention of their review of presentations that had pre-
ceded them; of their recognition of the progress made by this association;
and in the goal of eliminating livestock diseases. I do not choose to be an
exception. I, too, have made such a review. But in recognizing progress,
one notes a definite change that is of significant importance. The attitude
of "control" and "suppression" has been replaced by a militant, de-
termined attitude of eradication. For too long has the feeling of com-
placency played its part. For too long has the feeling of relaxation been
present. The determination to eradicate wherever possible will undoubt-
edly prove to be in the best interest of industry; to long-range budget de-
mands; and to the national welfare.

This year, 1963, marks the 46th year since the beginning of the Fed-
eral-State Cooperative Tuberculosis-Eradication Program. A look at the
record clearly shows the tremendous progress in this field. A closer
look, however, continues to bring into focus the repeated needs. At this
point I would like to commend the Committee on Tuberculosis, and its
Chairman, for their effort in regenerating a concerted interest.

Brucellosis on one hand, presents a very encouraging picture. We
have advanced a long way toward the goal of eradication in a relatively
short time. We speak freely of certified areas, and states. We point to
brucellosis free states, areas, and to some states now on the verge of
qualifying. This picture is surely one of which we can be justly proud.
But while we are pointing with pride, let us not forget that there are those
that are pointing at us—that are watching us. When funds were appropri-
ated for the accelerated brucellosis program, the year 1965 was estab-
lished as the target date.

After a great deal of study, on the part of members of this association
and responsible officials of the Agricultural Research Service, it was the
combined, considered opinion, that upon this date, every state would be in
the brucellosis certified status. This target date was supported by this
Association, and played a leading part in the testimony given to the Con-
gress to obtain their support for our requested increased appropriation.
If we meet this obligation, we shall forever be in a favorable position for
future requests. We shall be able to hold up this program as an example.
If we fail to meet this obligation, we shall then be in a position of attempting to explain why we failed. It is absolutely imperative that every effort be made to meet this target date, and that no one bear the responsibility of placing this Association on the defense.

When we look over the names of our various Committees, we find them to be more or less patterned one after the other. But there is an exception—a relatively new Committee, and a new Program. I invite your attention to the following name: "Committee on the Nationwide Eradication of Hog Cholera." This in itself eloquently describes the aim and objective.

For many years, this Association has sought to have the Agricultural Research Service take a more active part in the field of poultry diseases. Presently, after repeated requests on the part of this Organization, poultry diseases are being considered as a separate budget item, to be removed from the category of "Miscellaneous Diseases."

Three weeks ago, during the annual meeting of the New England Veterinary Medical Association a group composed of regulatory officials, poultry pathologists, and members of the poultry industry met to consider the means of establishing the New England States as a pullorum-typhoid free area. If this is to be considered, it follows that a determination and a declaration of policy should come from the Agricultural Research Service. Is eradication to be the goal in this field? Will they cooperate in establishing the definition of a "Free Area?" Will they encourage such a project at this time? Finally, will these areas be recognized?

During this, our 67th Annual Meeting, there is no doubt that program changes will be recommended, and will probably be adopted. It could well be that new programs will be recommended. When programs are adopted, or are changed, these actions are completed with the considered belief that they will be implemented. At times, in periods of relative emergency, as with program development, when lines are drawn to prevent, or to provide for restricted livestock movement, federal and state agencies are cooperatively committed. Each of these agencies attempt to carry out its respective commitment. Neither agency, however, is in a position to complete the job, for neither agency has the manpower required. We must, therefore, call upon the practicing, accredited veterinarian. The practitioner is gradually assuming the role as the very key in the enforcement of federal-state regulatory programs. He is the person who makes the required physical examinations; who certifies as to whether or not animals offered for shipment are free from symptoms of infectious, contagious or communicable diseases. He is the one who performs and certifies to required vaccinations. In many cases, he is the one who processes a certificate, that for all intent and purpose, complies with the requirements of the state of destination.

The importance of the practitioner is well recognized by everyone in the various regulatory agencies. But is this important role made known to him? I think that it is not.

Is he aware that controls are not necessarily needless red tape? Does he realize that in most cases, regulations are in effect that were
intended to be in the best interest of industry? Has he been made aware of the fundamentals of disease control and eradication? On the other hand, does he look upon regulations as the result of some overzealous bureaucratic agency?

We cannot afford to allow such an opinion to be created. If this does happen, it will be entirely due to the failure of every State Veterinarian, and his federal counterpart. Each of these officials should continually work toward a better liaison with their various State Veterinary Associations to create a better understanding and to eliminate violations through this liaison with these associations. The penalty of removal from an accredited list is not the answer. Aside from the few exceptions, it is difficult to imagine a person with the professional stature of the veterinarian intentionally or knowingly being an accessory in violating the sovereignty of our states. The solution then, comes right back where it belongs—to those responsible regulatory agencies. This is definitely our job.

It was my privilege to represent you in meeting with the Executive Committee of the National Association of State Departments of Agriculture, to assist in preparing budget requests to be presented to the Congress. I also represented this association at the annual meeting of the above organization held in Winston-Salem, North Carolina September 22-27. Two other members of your Executive Committee attended this latter meeting—namely, Dr. W. L. Bendix, Virginia, and Mr. Francis Buzzell, Maine. Resolutions governing poultry diseases, remodeling of the Beltsville facility, Screwworm eradication, backtagging as a means of identification, expansion of laboratory reference and consultation services, and the importation of foreign red meats were approved by their executive committee.

Other resolutions:
1. Requested that additional funds be allocated to expand the animal biologics testing service.
2. That research be conducted to determine the number of viable units of strain 19 to adequately increase protection to the desired level; and that the cause of some unfavorable reactions that occur in some calves vaccinated at the lower age level of four months be determined.
3. That the U.S.D.A. cooperate with the various states in developing a recognized state meat inspection service on an individual plant basis.

The one area that evoked the most discussion in this group was the inability of the Animal Inspection and Quarantine Division to fulfill its assigned duties because of the continued lack of financial support. Last year while this Association was meeting in Washington, D.C., the entire world was walking a tightrope, watching the situation in Cuba. In July of this year, we learned that the potential threat to our livestock industry was now a reality from that same area. Year after year, this association has repeatedly demonstrated to the congress our needs, and our pitiful lack of adequate defense and surveillance. Repeatedly the Congress has chosen to
ignore these requests and by so doing to gamble the fortunes of our livestock industry, and our national welfare. I hope that every member of this Association will make every effort at every opportunity to advise, and to alert their respective Congressional Delegate regarding this dangerous situation. It is their responsibility, and it is one that cannot continue to be ignored.

RECOMMENDATIONS

It has become custom, in recent years, for the members of your Committee on Programs and Policy to appear before the Congress in support of budget requests, in company with representatives of the National Plant Board, and representatives of the National Association of State Departments of Agriculture. There are some members of the latter organization that have expressed concern to me over the advisability of continuing this custom. I suggest to the incoming Chairman of the Program and Policy Committee that he pursue these discussions, and determine whether or not it is the wisest choice to continue joint congressional appearances.

The United States Livestock Sanitary Association for many years has made itself known in vast fields of research. We should, however, have a stronger voice in evaluating research, in establishing a priority, and in focussing attention to items that pertain to specific disease problems. Especially, we should increase our activity in participation in research at the Federal level. We have this opportunity, but we probably have not used it to its full advantage. I would recommend that our Committees, through their Chairmen, communicate with the members of the Program and Policy Committee as to the research needs in their various fields. Only in this manner will this Committee improve their position for discussion during their meetings with officials of the Agricultural Research Service.

During this past year, as in other years, various members have indicated to me a concern regarding the present membership of this Association, and in particular, that of the Executive Committee. Four years ago, during our Annual Meeting in San Francisco, an adopted change in our Constitution provided for two members to be elected representing the livestock industry from each of the regions as outlined by the United States Department of Agriculture Extension Service. It was believed at the time that this would provide an expanded opportunity for industry, through such representatives, to bring their problems and attitudes before this organization. By scheduling committee hearings in advance of this meeting, further opportunities are provided for industry to appear, and to voice their opinion. There is no doubt, that if advantage is taken of such opportunity, a great deal of help will be provided to guide our deliberations. It was pointed out to us last year that this plan has proven to be a valuable one, but to date has been somewhat limited.

The Executive Committee in the Annual Meeting in Washington, D.C. directed me to select a Committee on Ways and Means, to propose, if
necessary, changes in our Constitution to allow for further expansion of our Executive Committee. In compliance, I chose your elected officers.

It is their recommendation—

1. That such a Committee be continued.

2. That necessary changes be made in our Constitution and By-Laws to provide for expanded membership on the Executive Committee of this Association.

If this request is accepted, such membership will be limited to representatives of Associations that are National in scope and activity. The word National shall be interpreted as opposed to organizations that are regional or local.

Organizations requesting such membership shall make application to the Secretary-Treasurer, who shall so advise each of the elected officers. The elected officers shall present to the Executive Committee those applicants approved by them for membership, during the First Annual Meeting of said Committee.

Dues to be payable by these members shall be the same as those charged official members. Present voting requirements governing the acceptance of individual members shall apply.

At no time shall more than five (5) such applicants be presented for consideration.

Your Secretary-Treasurer, being a very realistic person, has foreseen that he will one day be replaced. In order to familiarize his successor with a first-hand knowledge of the complexity of his office, he has tape recorded his activities, his knowledge of meeting requirements, and the countless number of details that are involved. He has also attended Regional Meetings and has tried to keep the Executive Members informed. I trust you approve and will continue this practice.

In conclusion, I wish to extend my thanks in advance to the various Committee Chairmen for their part in contributing to what should prove to be a most interesting program, to those officials of the Agricultural Research Service with whom I have had the pleasure of discussion, to Doctor Hendershott for his many kindnesses, and to the membership of this Association who have afforded me the honor of having been able to serve.
PRESENTATION OF KEY TO PRESIDENT GRENNAN

R. A. Hendershott

Gentlemen: Some thirteen years ago, your Secretary designed a key emblematic of this Association and had a die made from which over the following years we have had a gold key tie holder made to serve as a memento of one's service to the Association.

The initial introduction of the presentation of this honor, took place at the time of our previous meeting in the Southwest, namely in 1950 at the Westward Ho Hotel at Phoenix, Arizona. All of the then-living past-presidents were presented with a key. Since then it has been my pleasure to present to each President a gold key of the Association which during recent years has been made into a modern tie holder.

It is my pleasure to make this presentation at this time. Doctor Grennan, if you will please come to the rostrum—- Over the years we have been, I think, very fortunate in the selection that has been made of the men who have been elected to the Presidency of this Association. One needs only to read the list of names of past presidents to realize that it really constitutes a review of the Who's Who of Regulatory Veterinary Medicine and Animal Disease Control and Eradication in North America.

Our present President has certainly lived up to the pattern set by those who preceded him to this high office—in fact, in some areas he excelled many of them. It has been a distinct experience for me to have served with him both prior to his term as President and during this, his presidential year.

Doctor Tom Grennan, it is a pleasure and an honor for me to present to you the tie clasp made from the likeness of the key emblematic of the United States Livestock Sanitary Association. On the reverse side is inscribed: "To Dr. T. J. Grennen, Jr. President 1963." I trust, sir, you will cherish this memento and wear it with the pride as do all of us who are so privileged, and may you have many more years of service in the work of this Association.

Dr. T. J. Grennan, Jr.: Thank you, Doctor Hendershott, and Members. It is an honor to receive this clasp and I assure you I shall wear it with pride.
MEMORIAL SERVICES
M. N. Riemenschneider

President Grennan, Distinguished Guests, Ladies and Gentlemen: We have now come to that part of the Program when we pause to pay our respects to our departed Colleagues. To the best of my information, the following members have passed away since our last meeting.

DR. B. T. SIMMS, SR. (AUB '11)

Dr. Simms died in Arlington, Virginia on September 26, 1963 of a heart attack. He was an Educator, Researcher, Chief of the United States Department of Agriculture Bureau of Animal Industry, and a former President of the American Veterinary Medical Association. Dr. Simms was a long time member of the United States Livestock Sanitary Association and a friend who devoted time and energy and was of assistance to our Association in bringing to light some important early history of our Association.

DR. FRANK H. BROWN (IND '10)

Dr. Brown, Indiana State Veterinarian from 1926 to 1933 passed away August 26, 1963. Dr. Brown, born in Hamilton County Indiana, practiced briefly at Knightstown after graduation from the old Indiana Veterinary Medical College in 1910. He served several years with the Federal Bureau of Animal Industry and joined Pitman-Moore Company in 1916 as a Field Consultant in Hog diseases. Since 1933, Dr. Brown had been in the veterinary supply business, most recently as a consultant with National Laboratories Corporation. He was honored during the recent AVMA Meeting at Miami as one of the veterinarians who had devoted more than fifty years to the profession.

EDGAR C. CLEVELAND, JR. (UP '13) 73
Cattaraugus, N.Y.
Died April 27, 1963

Dr. Cleveland started his practice in Cattaraugus in 1915, after teaching one year at the University of Pennsylvania. He continued to practice in Cattaraugus until his last illness. Active in civic affairs, he served as mayor from 1930 to 1934 and as president of the local school district for a time. Also active in his profession, Dr. Cleveland was a member of the Western New York V.M.A. and the New York State Veterinary Medical Society. He was a life member of the AVMA.
HOMER C. CURTIS (CVC '12) 76  
Polo, Illinois  
Died January 23, 1963

Dr. Curtis had been a general practitioner in Polo for many years. He was a member of the Illinois V.M.A.

LEROY DAVENPORT (MSU '43) 58  
Springfield, Illinois  
Died August 16, 1962

Dr. Davenport was Illinois State Public Health Veterinarian, and one of the first state public health veterinarians in the country. He was president of the Illinois Public Health Association in 1960-1961. He served as a consultant to the University of Michigan and to the University of Illinois.

BRUCE EDINGTON (OSU '12) 78  
Columbus, Ohio  
Died May 6, 1963

Dr. Edington was on the research staff of the Wooster Experiment Station for 32 years before his retirement. During the past 25 years, he had served on the staff of the College of Veterinary Medicine, Ohio State University, where he was chairman of the Department of Veterinary Research.

In 1960 he was awarded the Distinguished Alumnus Award by the College of Veterinary Medicine, Ohio State University. He was an honor roll member of the AVMA and a 50-year member of the Ohio State V.M.A. Dr. Edington was also a member of the Conference of Research Workers in Animal Disease and the U.S. Livestock Sanitary Association.

CLYDE C. FRANKS (CVC '11) 79  
Springdale, Arkansas  
Died May 1, 1963

Dr. Franks began his practice in Grimes, Iowa, then served for nine years as State Veterinarian. For many years he served as secretary of the Iowa V.M.A. He retired in 1951.

FRANK X. GASSNER (COL '37) 67  
Fort Collins, Colorado  
Died April 21, 1963

A noted endocrinologist at Colorado State University, Dr. Gassner was internationally known as one of the world's foremost authorities on infertility and reproduction problems of livestock. Much of his work was associated with similar problems in human medicine. He had also conducted extensive research on the effects of hormone implants and estrogen feed additives on growth rates of cattle.

From 1947 to 1960, Dr. Gassner was chief endocrinologist with the University's experiment station and professor of endocrine research. In
1960, he became professor of chemistry in the College of Science and Arts and professor of physiology medicine in the College of Veterinary Medicine.

Dr. Gassner received many honors from scientific societies, most recent of which was the Theodor Kitt medal awarded him in 1961 by the Veterinary Society of Munich for outstanding achievements in veterinary medicine. He was the first non-European to receive this award.

He was a member of many scientific and learned societies and was co-author of more than 100 technical articles published in scientific journals. He had presented about 70 scientific papers at conferences throughout the United States and abroad.

GEORGE W. GILLIE (OSU '07) 82
Fort Wayne, Indiana
Died July 3, 1963

Dr. Gillie, an immigrant from Scotland, had practiced in Fort Wayne, Indiana during his entire veterinary career. He was active in political and community affairs and was probably the only veterinarian elected to the U.S. Congress as a member of the House of Representatives.

He was elected to Congress in 1938 and served five consecutive terms. A member of the Committee on Agriculture, he became widely known for his fight to eradicate foot-and-mouth disease. He also held the distinction of being one of the first men elected to the Football Hall of Fame.

Dr. Gillie received the AVMA Award in 1953 for "Distinguished service to livestock health."

DR. WILLIAM A. HAGAN (KSU '15)

Dr. Hagan, Director of the National Animal Disease Laboratory, USDA, Ames, Iowa, died of a heart attack on February 1, during a transatlantic plane flight. Recognized as one of the outstanding veterinarians of his time, Dr. Hagan was nationally and internationally known as an eminent educator who possessed a broad understanding of practical as well as scientific veterinary medicine.

He was born at Fort Scott, Kansas in 1893. After teaching veterinary pathology for a year at Kansas State University, he joined the faculty at Cornell University's New York State Veterinary College in 1916 as an instructor of pathology and bacteriology. In 1926, he was made head of the department, and in 1932, at the age of 38, he was named dean. Dr. Hagan held this position for 26 years, earning the title of "Dean of Deans."

Dr. Hagan's services to veterinary medicine were world-wide. He was vice-president of the World Veterinary Association at the time of his death and had served for many years as the U.S. member of the Permanent Committee of the International Veterinary Congresses. He was the U.S. delegate to the 15th International Veterinary Congress at Stockholm, Sweden, in 1953, and to the 16th International Veterinary Congress at Madrid, Spain, in 1959. In 1958, he was chairman of a U.S. exchange
delegation to Russia. He was an honorary member of veterinary organizations in Great Britain, France, Sweden, and Greece, and was a member of many other professional organizations.

Dr. Hagan was the author of the outstanding textbook *The Infectious Disease of Domestic Animals* and more than 100 scientific papers and publications.

EDWARD E. HARNDEN (COL '23) 74
Stillwater, Oklahoma
Died May 26, 1963

A life member of the AVMA and the Oklahoma VMA, Dr. Harnden was professor emeritus of veterinary bacteriology at Oklahoma State University.

DR. JOHN G. HARDENBERGH (UP '16)

Dr. Hardenbergh, Executive Secretary of the American Veterinary Medical Association from 1941-1958, died Monday, February 11, 1963, at his home in Arlington, Virginia. Dr. Hardenbergh was born in Verkshire, New York. He received his V.M.D. degree from the University of Pennsylvania in 1916, and became a member of the AVMA in 1918. After graduation, he joined the Gilliland Laboratories, Inc. at Marietta, Pa., for two years, and then served with the U.S. Army Veterinary Corps from 1918-1920. He was affiliated with American Public Health Association, taught part-time at the University of Pa., and was the Secretary of the Veterinary Medical Association of N.J. In 1959, Dr. Hardenbergh was presented the Annual AVMA award in recognition of his distinguished service as executive secretary for 18 years as well as his countless contributions to the Veterinary profession as a whole. From 1958 until his death, Dr. Hardenbergh served as a consultant to the AVMA.

HAROLD O. PETERSON (WSU '36) 57
Albuquerque, New Mexico
Died July 10, 1963

Dr. Peterson was director of the Animal Disease and Parasite Research Laboratory of the Agricultural Research Service, United States Department of Agriculture, at Albuquerque, New Mexico. Dr. Peterson enjoyed a national reputation as a research scientist in the field of animal parasitology.

In his capacity as a parasitologist with the United States Department of Agriculture, he was first attached to the Food and Drug Administration, the Zoological Division of the Bureau of Animal Industry, and later to the Animal Disease and Parasite Research Division, Agricultural Research Service, of which he became director in 1954.

Dr. Peterson's numerous publications included thirty journal papers and technical bulletins in the field of parasitology and veterinary medicine. He was a member of many professional organizations.
MEMORIAL SERVICES

CHARLES L. VICKERS (GA '51) 41
Columbus, S. Carolina
Died April 23, 1963

Dr. Vickers was a federal veterinarian, serving with the USDA in connection with Clemson College Extension Service.

I request all present to arise and remain standing for a moment of silent prayer for the peaceful repose of the souls of our departed Colleagues.

SILENT PRAYER

Thank you for your respectful participation.

In memory of the departed members of this organization, I would like to read a poem, which to me, symbolizes one of the many virtues of these men.

BROTHERHOOD

A little kindness every day
While we're at work or at our play!
A little smile, a little song,
A word of cheer when things go wrong.
To help a brother on his way!

It's such a little thing to do,
Yet means so much to me and you!
Let's not forget that little smile,
That little kindness all the while,
Which gives us joy and courage, too!

A little kindness... 'twas the Plan
To help us all since time began!
The only heaven that we can know
Along life's pathway as we go,
Is love bestowed by man to man!

- Author unknown

To evaluate the life and work of these men, one would need to review many contributions to the science of disease control, to the livestock industry, to public health, and related fields. These contributions in no small way have contributed to the success of Agriculture, especially Animal Industry, which is the most productive in the history of the world, providing this Nation with an abundant food supply. Let us be thankful for these contributions to our way of life and may we never forget how fortunate we are to be a Nation that does not go to bed hungry.
I am sure all of us feel a keen sense of personal loss that we will not have the presence and counsel of these Colleagues, but let us be thankful for the privilege of having known them; for the opportunity of working with them; for the contributions they have made in making this a better place to live; for the goals and ideals they have passed on to us.

Let us memorialize them this day by putting forth our best efforts to ever preserve the ideals they have passed on to us and ever go forward adding to their accomplishments. We know they will always be remembered for themselves as well as their work.
CONSTITUTION AND BY-LAWS
OF THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME
The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE
The purpose of this Association shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of the laws, regulations, policies and methods pertaining to milk and meat hygiene, and to the prevention, control and eradication of transmissible livestock diseases; to maintain co-ordination among the various livestock regulatory organizations, and to serve as livestock sanitary science clearing house between this Association and the following: The livestock owner, the livestock sanitarian, the milk and meat hygienist, the veterinary practitioner, the transportation and stock yard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "livestock" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP
There shall be three kinds of members—Official and Individual and Non-Voting Junior.

OFFICIAL MEMBERSHIP
The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, Puerto Rico, the Virgin Islands and Los Angeles County, California shall be eligible to official membership in this Association and be represented on the Executive Committee by the livestock sanitary executive official.

INDIVIDUAL MEMBERSHIP
Any person engaged in livestock sanitary work for Federal, provincial, state, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.

JUNIOR NON-VOTING MEMBERSHIP
Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.
ARTICLE IV—MEETINGS

The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS

The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Secretary-Treasurer, and an Executive Committee.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various States, the Director of Livestock Regulatory Programs of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive regulatory officer of Cuba, Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, the elective officers of this Association and eight delegates at large representing the livestock industry including poultry.

No more than two delegates from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast; consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; the North central, consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; the Southern, comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the Western district consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry, including poultry. No more than two (2) delegates at large shall be elected from each of the four designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies. All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee. The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.
The Executive Committee shall cause to be audited annually or oftener if deemed necessary, the receipts and disbursements of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.

That, with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining assets thereof shall be contributed for utilization in the advancement and research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain.

ARTICLE VI—PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee and the Secretary-Treasurer and the Chairman of the respective committees shall constitute the Program Committee. It shall be the duty of the officers of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII—DUTIES OF OFFICERS

1. President: It shall be the duty of the president to preside at all meetings of this Association; to appoint all committees excepting the Executive and Officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon the written request of five members of the Executive Committee. The president shall be an ex-officio member of all committees.

2. President-Elect: The president-elect shall be chairman of the Executive Committee. In the absence of the president, he shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the president he shall perform all duties of the president. He shall be an ex-officio member of the Executive and Program Committees.

3. First Vice-President: The first vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president and president-elect. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect. He shall be an ex-officio member of the Executive Committee.

4. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, president-elect and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect and first vice-president. He shall be an ex-officio member of the Executive Committee.
5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association.

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.

ARTICLE VIII—AMENDMENTS

The constitution of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting and further provided that the amendment has received the approval of the Executive Committee.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary-Treasurer.
President's Address
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers and eight members to Executive Committee.
Adjournment.
A suspension of the By-laws may be made by a two-thirds majority for the purpose of changing the order of business or to facilitate important business.

ARTICLE II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary-Treasurer. The Application shall give the name,
occupation and address of the applicant and shall be accompanied by a fee of five dollars ($5.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary-Treasurer, who shall in turn submit them to the Executive Committee.

An individual member may be expelled for cause by the Executive Committee.

ARTICLE III—MEETINGS

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association.

The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV—QUORUM

Twenty-five members of the Association shall constitute a quorum.

Twenty members of the Executive Committee shall constitute a quorum.

ARTICLE V—DUES

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association.

The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association.

The dues for official memberships shall be one hundred dollars ($100.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
PROPOSED AMENDMENT TO THE CONSTITUTION

T. J. Grennan
Providence, Rhode Island

The Constitution and By-Laws of the United States Livestock Sanitary Association shall be further amended as follows:

1. Article III - Membership
   Line 20 shall be amended by adding "and Association."

2. Between Line 36 and 37, the following shall appear.
   "Association Members"
   "Any person, duly authorized to represent any organization that is national in scope and activity, that is directly concerned with the interests of this Association as outlined in Article II - Purpose - may be elected to association membership."

3. Between Line 74 and Line 75, the following shall appear.
   "The elected officers, at the first regularly scheduled annual meeting of the Executive Committee, shall have the authority to place before said committee applications for Association Members. At no time shall more than five such applications be presented for consideration. Voting procedures, as outlined to accept or reject applications for individual membership shall apply. Dues for association memberships shall be payable and subject to the same terms as are provided for official memberships in accordance with the provisions of Article V - Dues - of the By-Laws."

"By-Laws"

1. Article II - Applications for Membership
   Between line 180 and 181 the following shall appear:
   "Applications for association membership shall be made in writing to the Secretary-Treasurer on an appropriate form prepared by him. In turn, notice of receipt of such applications shall be provided each of the elected officers."
Mr. President, Ladies and Gentlemen, Members and Guests of the United States Livestock Sanitary Association: Welcome to this, our Sixty-seventh Annual Meeting. I am certainly pleased with the great number of registrants to date—somewhat more than 300. I made the statement that if we had 200 present it would be a decided success; we have already passed that number.

I do not see our good friend, Doctor F. L. Schneider, at this moment but certainly his heart must glow with the knowledge that so many have journeyed to Albuquerque to do him honor, for he more than any other one person is responsible for the 67th Meeting being held here this week.

While I was not what one might call enthusiastic about meeting so far from our Constitutional home, Chicago, my lack of enthusiasm was in no way related to the respect and warm feeling I have and will always maintain for my good friend and associate, Doctor F. L. Schneider. I am glad we are here and am certain we shall carry away fond memories of this meeting which bids fair to measure up to the high standard of its predecessors.

The Sixth Annual Conference of Veterinary Laboratory Diagnosticians completed two days of an excellent meeting. They have proved to be an asset to our Association and their program and papers have added materially to our Proceedings.

I continue to have difficulty in obtaining copies of papers from contributors to our program—the last one in was received May sixth—which really fouled us up this year. This needless procrastination has an ever widening effect which this year resulted in the proceedings being mailed quite late and in turn delayed the delivery of reprints to such an extent that many of them remain unpaid. Some of these accounts would have paid out of fiscal year monies which probably reverted to the general treasuries in some instances on June 30. Our Treasurer Report reflects the trouble caused by failure of persons to meet our deadline for delivery of papers in that for the first time in my experience we have an accounts receivable of $1799.

At the Sisty-sixth Annual Meeting, I expressed the desire to be invited to attend regional meetings of our Executive Committee and am pleased to tell you that Doctor John Quinn, Chairman of the North Central group, invited me to attend their meeting in April and to tape record their remarks. Likewise, Dr. C. E. Kord invited me to attend the Southern States meeting at Ellender Agricultural Center in Nashville, Tennessee in May and tape record their meeting and also the second day joint meeting with Federal Veterinarians of the area.
Through the experience gained in tape-recording these meetings, I plan to use my tape recorder at this meeting and believe through its use to be able to save a couple of hundred dollars of cost. I have taped all presentations of the Conference of Laboratory Diagnosticians. It is my hope to develop a library of tape recordings of our meetings.

This year we changed printers and contracted with a concern in Ann Arbor to print the Sixty-sixth Proceedings at a savings over previous years and I think at an easier reading-type face.

As a member of the Committee on Program and Policy, several meetings were attended.

Recently, we have lost a particularly valued friend and associate in the passing of Doctor Bennett T. Simms, Sr. It was largely due to his interest and effort over the past decade that I was able to assemble accurate information relative to the Officers and places of meeting of the early years of our existence as an Association. It was Doctor Simms who obtained copies of the 1903-1904-1906-1907 reports and loaned them to me for reproduction. More recently he sent me a copy of the Third Annual Meeting which was reproduced on yellow pages and included in the Sixty-sixth Annual Proceedings. We were in constant touch with each other and he was always looking for copies of missing issues such as 1898-1899-1900-1901. These latter four are bound in one cover and were in the library of the United States Department of Agriculture, but someone has them out and not returned them. So far as is known, these are the only copies in existence. Truly we have lost a good friend.

Following is the report of the Treasurer and Certified Public Accountant.
REPORT OF THE SECRETARY-TREASURER

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR THE PERIOD FROM OCTOBER 9, 1962 TO SEPTEMBER 30, 1963

Cash Balance, October 10, 1962:
First Trenton National Bank, Trenton, N. J. $ 219.20
Trevose Savings and Loan Association, Morrisville, Pa. 5,552.37 $ 5,771.57

Increased by Cash Receipts:
Individual Dues 5,424.76
Official Dues 5,350.00
Proceedings 1,693.53
Foreign Annual Disease Handbook 175.49
Brucellosis Facts ("What is Known about Brucellosis") 250.00
Reprints 2,083.71
Registration Fees 3,570.00
Grant "World Health Organization" 1,000.00
Interest on U. S. Treasury Bonds 800.00
Interest on Trevose Savings and Loan Account 255.68
Total Cash Receipts 20,603.17

Decreased by Cash Expenditures:
Meeting Expenses 969.47
Printing and Stationery 8,121.07
Salary 6,500.00
Travel 2,041.33
Communications 750.43
Rent 360.00
Insurance 182.80
Electricity 82.86
Interest 20.00
Repayment of Loan (Trevose Savings and Loan Association) 4,000.00
Animal Virus Classification (World Health Organization Grant) 900.00
Miscellaneous 207.63
Total Cash Expenditures 24,115.59

Cash Balance, September 30, 1963:
First Trenton National Bank, Trenton, N. J. 259.15
Trevose Savings and Loan Association, Morrisville, Pa. 2,000.00 $ 2,259.15

Note: The attached letter is an integral part of this exhibit.
R. A. HENDERSHOTT
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS
FOR THE PERIOD FROM OCTOBER 9, 1962 TO SEPTEMBER 30, 1963

Revenue:
Total Cash Receipts $20,603.17
Accounts Receivable 1,799.33

Expenditures:
Total Cash Expenditures 22,402.50
Less: Repayment of Loan 4,000.00

Net Revenue from Operations for Fiscal Period $2,286.91

NET WORTH - SEPTEMBER 30, 1963

Accounts Receivable $1,799.33
Balance, First Trenton National Bank, Trenton, N. J. 259.15
Balance, Trevose Savings and Loan Association, Morrisville, Pa. 2,000.00
U.S. Treasury Bonds, 4%, Due February 15, 1980 20,000.00
Furniture and Fixtures 640.00
Net Worth, September 30, 1963 $24,698.48

ANALYSIS OF CHANGE IN NET WORTH

Net Worth, October 9, 1962 $22,411.57
Increased by:
Net Revenue from Operations for Fiscal Period Ended September 30, 1963 2,286.91
Net Worth, September 30, 1963 $24,698.48

Should anyone have any questions regarding any items in this report I shall be pleased to answer them. In closing I wish to state that it has been a pleasure for me to serve you. Thank you for your kind attention.
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS


PROPOSED FEDERAL-STATE HEALTH CERTIFICATE

We respectfully request the United States Department of Agriculture, Animal Disease Eradication Division to determine if an official state health certificate form that provides all essential requirements of the proposed federal health certificate could be used in meeting the requirements of the federal regulations on livestock moving in interstate commerce.

Your Committee urgently requests that each state adopt an official health certificate form recommended by the United States Livestock Sanitary Association in 1954, in 1962 and recommended by the United States Department of Agriculture in 1963. It is further recommended that each state review and improve their system of control of official health certificates to assure proper use and issuance so as to be acceptable to both state and federal agencies.

Your Committee, again, recommends that the United States Department of Agriculture, Animal Disease Eradication Division adopt and use the proposed uniform health certificate in lieu of the federal form 48 when livestock are consigned to other than approved slaughtering establishments, approved quarantined feedlots or other approved markets.

INTERSTATE LIVESTOCK HEALTH REGULATIONS

Fifty individual states have livestock health requirements in addition to federal regulations pertaining to the movement of livestock in interstate commerce. This impractical situation has gradually come about over a long period of time. In order to very graphically call to the attention of the United States Livestock Sanitary Association, the Committee on Laws and Regulations listed the variations found in state health requirements in its 1961 report.

As forcibly as the Committee can, it wishes to insist on the full attention of every member of the United States Livestock Sanitary Association on this existing situation and request its full concern and study of the problem.

Your Committee sincerely believes that, in this day of accomplishment in veterinary medical science, there is no longer any justifiable reason why a person wishing to ship livestock in the United States should be faced with fifty-one sets of livestock health regulations. Therefore, we are proposing that the Committee on Laws and Regulations have as its
objective the development of a Recommended Uniform Livestock Inter-state Health Regulation. The final result being that there will be one uniform set of regulations acceptable to all states and the federal government. The regulations would be based upon the best scientific facts available and would assure maximum control of animal diseases in the interstate movement of livestock.

The procedure proposed for the development of such a recommended regulation would be for this Association, through the Committee on Laws and Regulations, to start immediately to prepare the recommended regulation, calling upon all scientific advice available in the United States to formulate specific portions of the regulation, obtaining advice and assistance from all segments of the livestock industry and holding annual hearings at this Association's meeting for review and consideration of each portion of the regulation. It would be the aim to develop such a regulation so basically sound and so completely acceptable that there would be no reason for its not being adopted by either federal or state agencies.

Your Committee realizes this is a big order, but they are emphatic that it must be done as soon as possible. There is no better organization in existence to undertake and accomplish this task.

FOREIGN DISEASE IMPORT REGULATIONS

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

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

PROPOSED MEAT INSPECTION LAWS

The Committee reaffirms the policy statement adopted in 1962.
REPORT OF THE COMMITTEE ON NOMINATIONS

J. G. Milligan, Montgomery, Alabama, Chairman; W. L. Bendix, Richmond Virginia; F. G. Buzzell, Augusta, Maine, A. P. Schneider, Boise, Idaho

The Committee on Nominations would like to say that we feel that the Association was wise in its choice of President for the coming year. It has been our hope and ambition that we could come up with the best slate of nominees for office for the coming year.

We would like to propose the following officers to assist Doctor Rosner.

President-Elect - Dr. John W. Safford, Helena, Montana
1st Vice-President - Dr. C. L. Campbell, Tallahassee, Florida
2nd Vice-President - Dr. Grant S. Kaley, Albany, New York

We are also charged with the responsibility of naming nominees to the Executive Committee. This has been very difficult because we realize that throughout the years certain nominees presented by us and elected by this Association have not attended. I do not know if your Committee is in a position to screen the field well enough to come up with a recommended slate that would allow us to get people to attend. We are most grateful to those persons who have attended in the past. We would like to present the following slate:

From the Southern Region
Mr. J. B. Finley, Encinal, Texas
Mr. James Nance, Alamo, Tennessee

From the North-Central Region
Mr. J. R. Bishop, Atlanta, Indiana
Mr. Ward Van Horn, Buffalo, South Dakota

From the Northeast Region
Mr. Chester Pilch, Hazardville, Connecticut
Mr. George E. Coleman, Jr., Brunswick, Maine

Now we come to the Western group who have faithfully attended meetings and we thank them.

From the Western Region
Mr. O. H. Timm, Dixon, California
Mr. A. O. Wilson, St. Xavier, Montana

These are the names of those your Committee on Nominations would like to present to you for your consideration.

President Grennan: Thank you, Doctor Milligan. Gentlemen, we shall entertain nominations from the floor.
After a pause, the Chair recognized Doctor Glen B. Rae of Oregon who moved that the nominations be closed and the report of the Committee be accepted and that the chair cast a ballot for the election of the proposed slate. The motion was duly seconded.

Dr. Grennan cast a unanimous ballot for the slate presented by the Nominations Committee.

Doctor Milligan escorted Doctor Rosner and Mr. Buzzell escorted Doctor Safford. Both Doctors Campbell and Kaley were absent. Doctor Campbell had left the previous evening to attend an important meeting on Equine Peroplasmosis and left a note explaining his absence. Both Mr. Timm and Mr. Wilson were absent.

**Doctor Safford:** Gentlemen, it is indeed an honor to be elected President-Elect of this illustrious organization. On behalf of the people of Montana I accept your election and sincerely hope the next two years that my efforts will be worthy of this election. Thank you. Applause.

**Doctor Grennan:** I wish to express my deep thanks to all those I had a chance to work with and wish Len Rosner a lot of success and now turn this duty over to him. Applause.

**Doctor Rosner:** Gentlemen, I feel most honored that you have elected me your President for the next year. I am grateful and I want you to know I am aware of the tremendous responsibility that this office carries. I know I must call on many of you for your advice and assistance and I hope you will bear with me. Thank you very much.

**Doctor Grennan:** Gentlemen, if there is no further business I would like to call this meeting adjourned, and remind members of the Executive Committee that the final meeting of that body for this meeting will take place immediately in this room.
With the development of the sulfonamides in 1935 and antibiotic, starting with penicillin in 1941, the foundation for great advances in pharmaceuticals was begun. Most of these products are now common knowledge. The more recent developments were brought out in the previous reports.

The process by which an effective remedy is developed for the treatment of a specific infection may be divided into four parts:

First is the identification of a particular agent, protozoal, viral bacterial cause of the disease process. This stage has usually been undertaken by publicly or privately endowed institutions.

Second - the study of the etiological agent to learn its life cycle and its relations to the host. The complexity of this study varies greatly with the agent concerned. Experimental chemotherapy is concerned with the convenient propagation of the agent. This stage may be carried out in microbiology, parasitological or zoological laboratories of non-commercial institutions. The adaptation of the agent to use as a screening tool for pharmaceuticals is often carried out by industrial firms.

The third stage consists of the making and selection of chemical compounds and the testing of their effect upon the organism or agent in vitro and/or the effect in vivo. This third stage may be subdivided into an initial stage to detect activity and followed by a development stage for large-scale production of the product and field evaluation under both controlled and clinical conditions. This stage is carried almost entirely by industrial firms. It requires extensive work by many chemists and biologists for years. Field testing is done by experiment stations and commercial operations under the supervision of industrial and government scientists.

The fourth stage, working with what is now an effective pharmaceutical, is to study the mode of action and fate of the compound. This stage is largely biochemical; the general cell biology is better understood. This type of research tends to have more opportunities as techniques improve.

This brief outline shows that success in pharmaceutical therapy requires a broad outlook, embracing many different disciplines. A good understanding of the life history of a disease agent and the ecology of modern husbandry are indispensable to veterinary pharmaceutical research.

Federal regulations regarding pharmaceuticals for livestock and poultry use, following the food additive amendment of 1958 have resulted in requirements that caused some confusion and unusual delays in obtaining clearance for products to be used by the livestock industry and
veterinarians. Some applications have been considered by three different sections each with a different finding and interpretation; however, recent administrative changes have brought about some improvement in this situation.

The 1962 "Drug Amendment" resulted in regulations which became effective in February, 1963. The regulations prohibited the sale of livestock and poultry which have received a new drug unless clearance was obtained from F.D.A. prior to sale. Permission to market the drug is predicated on data presented involving toxicity and metabolic fate of the compound concerned. These requirements, although imposing, can be complied with at an increased cost to the industry.

Pending legislation in the form of the "Roberts Bill" HR 7247 and three other identical bills introduced in the current session of Congress, hearings on the bill were held by the Committee on Interstate and Foreign Commerce last September 17-18-19.

Some of the major sections provide for the elevation of the Veterinary Section, now operating under the Medical Bureau, to the status of a Bureau:

- A provision to consider all antibiotics for Veterinary use as non-certifiable.
- A provision for master files of Manufacturing Procedures of Medicated Feeds and others was considered.
- Testimony for the bill was heard from the American Feed Manufacturers Association, The Animal Health Institute, Representatives of the Animal Husbandry Department of Iowa, The Poultry Industry, the Cattle Industry and others.
- Testimony against the bill was presented by the American Veterinary Medical Association and a representative from Missouri.

Your Committee on Pharmaceuticals makes no recommendation concerning the position the United States Livestock Sanitary Association should take with respect to HR 7247 but wishes to inform the membership of developments in the pharmaceutical field.

New products of interest are Sevin and Civdin, both trade names, which may be used as pesticides and pose no milk or meat residue problems.

The use of various sulfonamides for the treatment of Atrophic Rhinitis when caused by a number of causative agents has been reported by Iowa workers.

The food additive compendium published by the Miller Publishing Company of Minneapolis provides a ready source of information concerning pharmaceuticals used as food additives.
REPORT OF THE COMMITTEE ON PROGRAM AND POLICY

L. A. Rosner, Jefferson City, Missouri, Chairman; W. L. Bendix, Richmond, Virginia; T. J. Grennan, Jr., Providence, Rhode Island; R. A. Hendershott, Trenton, New Jersey; J. A. McCallam, Washington, D.C.; J. F. Quinn, Lansing, Michigan; J. W. Safford, Helena, Montana; C. L. Campbell, Tallahassee, Florida

Mr. Chairman and Members of the Association: The Committee on Program and Policy as in previous years met in Washington, D.C. in March and in June of this year for the purpose of reviewing all of the disease control and eradication activity of the Agricultural Research Service and to review the appropriation request for the 1963-64 and the budget request for 1964-65.

As Chairman and on behalf of this Committee, I would like to express our appreciation to the various staff members of the Agricultural Research Service who gave so unstintingly of their time and effort to the Committee; I want you to know they have been most cooperative with this Committee both last year and certainly this year, and I think you should know that.

As in the past, our review of the several cooperative disease control and eradication programs and the budget item request for funds was most detailed and thorough. Likewise, our recommendations reflect the many recommendations of the standing committees of this Association and to some lesser extent the thinking and the recommendations of our member conferences or regional group meetings.

Since the 1962 report of this Committee was most comprehensive and detailed, a further detailed report or discussion of programs and activities is purposely avoided in the interest of brevity and to avoid being repetitious with the recommendations of past years. With the recommendation that you again carefully review the 1962 report of this Committee, this report should be carefully studied for it will give you an insight into the Federal-State Cooperative Activities and I believe that this report reflects the current viewpoints and recommendations of this Association.

The Recommendations of this Committee on specific budget items for the 1964-65 fiscal year are essentially as for last year with these exceptions. (1) The Committee recommended an additional $750,000 to provide the additional personnel needed for the greater utilization of the existing facilities at Plum Island; (2) $1,500,000 for the addition of a large animal handling facility at Plum Island; (3) There is an additional recommendation of a million dollars for working over the sewer, water, electrical systems of the Plum Island plant.

Now for the National Animal Disease Laboratory at Ames, Iowa—the Committee feels that an additional two million dollars is needed and was recommended to complete the facilities at Ames. This is badly needed for the research that should be initiated. The Agricultural Research
Service recognizes the need for more basic research and their budget contained requests for additional funds for establishments of regional laboratories. Obviously, this involves a very considerable amount of money—as I recall, roughly four million dollars for each facility. It was the thinking of this Committee that there should be a very detailed study made of the facilities available in colleges in the United States and that these should be fully utilized prior to the establishment of new research laboratories.

This does not mean that the Committee is opposed to the request items for research; the Committee does feel that we should, however, make fuller use of those facilities in the various colleges and research stations throughout the nation that may now be available. This would allow us to get on with some of the urgently needed research.

INSPECTION AND QUARANTINE

As you know, this Association has for years been seriously concerned with the inadequacy of our control over the introduction of foreign animal diseases. Previous studies by a Committee from this Association and by the Committee on Program and Policy reflects the inadequacy of our port-of-entry facility. As the report of 1962 indicated, we have been amazed at what the Animal Inspection and Quarantine people have been doing and the conditions under which they work. It has been said before and I would again say that these port facilities have been woefully inadequate; in some instances they hardly exist. Within the past year there have been some funds provided and new facilities; however, there is much more needed both in facilities and personnel to provide the protection that is needed.

In Import-Export Work, the Committee recommended an added $1,800,000; this amount is in addition to that provided for in the 1964 budget which is now before the Congress. Of this sum, one million is needed for the construction of adequate facilities at Miami, Florida and at Los Angeles; the remaining $800,000 would provide for the added personnel that is needed to staff these and to extend AIQ supervision over the importation of animals, products, et cetera.

BIOLOGICS TESTING

Another area within the responsibility of Animal Inspection and Quarantine that has been historically of concern to this Association is that of the Biologics Licensing Section and their capability for testing biologic products. Some additional funds were provided for the 1963-64 year and the results obtained from the use of this money have been most satisfying; however, they emphasize the need for greater activity in the area and more adequate biologic testing work. I believe that so far they have brought the level of this work to about 15 percent and it is our thinking that this should be stepped up to about 45 percent of the biologic products. To date, their testing has revealed that some of the products are practically worthless. We maintain that our Livestock Industry and the Veterinary
Profession are certainly entitled to better products and the Government cannot condone the licensing of those that may fall into the worthless or harmful category. So the Committee recommended that the budget request for this work be increased by some $300,000.

**TUBERCULOSIS ERADICATION**

In the area of Tuberculosis Eradication, I need not tell you that we have stood still for a number of years. The Committee feels that in view of the change of the Uniform Rules and Regulations which provide for a better and more intensive approach to this puzzling eradication effort, an additional two million dollars is needed and was so recommended.

The Committee recommended funds for the Brucellosis Eradication Program to continue the work at the present rate.

With regard to Scabies, some five hundred thousand dollars was available for 1963-1964 fiscal year and the Committee requested an added fifty-eight thousand dollars ($58,000) for this work. It is the feeling of your Committee that given sufficient funds for a period of two years, this disease could be eradicated and expenditures for Scabies Eradication could be radically decreased or eliminated.

**MEAT INSPECTION**

In the area of Meat Inspection work, there are many problems. The Committee recognizes that the federal meat inspection service must necessarily extend its service to a number of plants that qualify and have made request for this inspection. The requests for this service by new plants in heretofore uninspected areas always exceeds annual budget provision. In order to provide as far as possible some of this demand, the Meat Inspection Division has resorted to employment of its staff on overtime, the extra time cost being borne by the Packer. In addition, mandatory pay increases within the range of pay of the employee must be met out of the sum appropriated. Currently, this Division operates on a budget of about 26 million dollars. Your Committee feels that the cost of all meat inspection service should be borne by the Government, that sufficient monies should be appropriated to obviate the necessity for overtime work at the Packers' expense, that funds should be sufficient to more nearly take care of the ever-increasing demand for Federal Meat Inspection. Having in mind the importance of federal meat inspection to the health and well-being of all of our people, the Committee recommended that the sum of thirty-three million dollars be requested in the budget for 1964-65 fiscal year.

**HOG CHOLERA ERADICATION**

For Hog Cholera Eradication, the Committee recommended an increase of $1,500,000 over the request of four million dollars.

Gentlemen, those are essentially the Committee's recommendations and I thank you for your patience. Thank you.
REPORT OF THE COMMITTEE ON RABIES


RABIES INCIDENCE AND TRENDS - CALENDAR YEAR 1962

The total number of laboratory confirmed animal rabies cases reported for calendar year 1962 was 3,727, representing an increase of 257 cases over the total number of confirmed cases reported during the previous calendar year. Thus, the continual decline which occurred between 1946 and 1960 has now levelled off. The most important factor in the 15-year decline of total reported cases has been the effective control of canine rabies: dog rabies cases now have been brought to a relatively low residual level. Further reduction in the number of cases of animal rabies now depends on the development of rabies control measures for wildlife.

Of the slightly over 3000 counties in the United States, 998 reported one or more cases of animal rabies. This compares with 934 reported for 1961, and 887 for 1960.

The numbers of confirmed cases reported for 1962 by type of animal included 565 dogs; 232 cats; 614 farm animals; 594 foxes; 1,449 skunks; 157 bats; and 114 cases in other types of animals. A slight decline occurred in dogs, with a drop of 29 between 1961 and 1962. The total of 565 canine cases is the all-time low reported for the entire country.

In 1962, there was a slight decrease of rabies in foxes. There were 594 cases in foxes compared with 614 cases during calendar year 1961. The southeastern states, which long have been areas of high fox rabies, are now virtually free of it.

Reported skunk rabies cases increased from 1254 cases in 1961 to 1449 cases in 1962. The increase of skunk rabies in Ohio was explosive; the number of confirmed cases rose from 28 in 1961 to 310 in 1962.

An epidemic of rabies in raccoons has been moving northward in Florida for several years. This now has crossed Florida’s northern boundary involving south Georgia.

The number of rabid bats in 1962 was 157 as compared to 186 for the previous year. These cases came from 26 States. California, Texas, Florida and Oregon accounted for 90 of these.

The New England States remained rabies free in 1962 with the exception of three reported bat cases in Massachusetts and one reported fox case in Maine. Massachusetts reported one bat case for 1961 which was the first reported case of animal rabies in the State of Massachusetts since 1949. The fox case reported in Maine for calendar year 1962 is the first reported case of animal rabies in Maine since 1951.
STATES WHICH HAVE REPORTED BAT RABIES WITH DATE OF FIRST ISOLATION

CASES OF RABIES IN WILD AND DOMESTIC ANIMALS 1953-1962

Source USDA, ARS and USPHS, CDC
There were 23 States in which there was an increased incidence in total rabies cases over the previous year: Alaska, Arizona, California, Florida, Georgia, Idaho, Indiana, Kentucky, Maine, Massachusetts, Minnesota, Nevada, New Jersey, New York, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Dakota, Tennessee, West Virginia and Wisconsin. Of these, there were three States in which the increase was by more than 50 cases, Indiana, Minnesota and Ohio. The principal problem in each of the three States has been one of skunk rabies. Texas again showed the highest incidence in the country with a total of 531 cases of animal rabies for 1962. Ohio showed the greatest increase with 309 more cases for 1962 than for the previous year.

In 17 States there was a decrease in the number of cases reported: Alabama, Arkansas, Colorado, Illinois, Kansas, Louisiana, Maryland, Michigan, Mississippi, Missouri, Nebraska, New Mexico, North Carolina, Texas, Utah, Virginia and Washington. In Missouri, Texas and Virginia the decrease was by more than 50 cases. Texas showed the greatest decrease with 127 fewer cases in 1962 than in 1961.

RABIES TRENDS BY UNITED STATES PUBLIC HEALTH SERVICE REPORTING REGIONS - 1962

New England
(Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, and Connecticut)

The only cases of animal rabies reported during 1962 were three bat cases in Massachusetts and one fox case in Maine.

Middle Atlantic
(New York, New Jersey, and Pennsylvania)

New York's incidence increased from 92 cases in 1961 to 107 cases during 1962, an increase of 15 cases. New Jersey showed a slight increase, reporting 10 cases during 1962 as compared with seven cases the previous year. Pennsylvania reported an increase from 14 cases to 58 cases. Thus, this reporting region had an increase of 62 cases.

East North Central
(Ohio, Indiana, Illinois, Michigan and Wisconsin)

Ohio's incidence increased from 77 cases in 1961 to 386 cases in 1962, an increase of 309 cases. This was the greatest increase reported in the entire country. Indiana showed an increase from 109 cases to 196 cases. On the other hand, Illinois and Michigan showed slight decreases in reported cases; Illinois from 163 to 133 and Michigan from 80 to 48. Wisconsin showed an increase of 18 cases over the previous year.
West North Central
(Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska and Kansas)

This reporting area showed an increase of 41 cases in 1962 with Minnesota, North Dakota, and South Dakota showing an increase, Missouri, Nebraska, and Kansas showing a decrease and Iowa remaining the same for 1961 and 1962 with 349 cases.

South Atlantic
(Delaware, Maryland, District of Columbia, Virginia, West Virginia, North Carolina, South Carolina, Georgia and Florida)

This reporting area showed a decline of 29 cases in 1962 with only West Virginia, Georgia and Florida showing increases.

East South Central
(Kentucky, Tennessee, Alabama and Mississippi)

This reporting area showed an increase of eight cases for 1962. Kentucky and Tennessee showed increases and Alabama and Mississippi showed decreases.

West South Central
(Arkansas, Louisiana, Oklahoma and Texas)

A decrease of 194 cases for this reporting area. This [decrease] was due largely to the decrease of cases in Texas from 658 to 531. Texas still had the greatest incidence in the country.

Mountain
(Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah and Nevada)

There was a decline from 68 cases in 1961 to 48 cases during 1962, with only Idaho, Arizona, and Nevada reporting slight increases.

Pacific
(Washington, Oregon, California, Alaska and Hawaii)

This reporting area showed an increase of 42 cases from 1961 to 1962. The bulk of the cases was reported from California. Hawaii remained rabies-free.

HUMAN RABIES DEATHS - 1962

Two human rabies deaths were reported in the United States for calendar year 1962. The first case occurred in a four-year-old male who died of rabies in Cameron County, Texas, on July 24, 1962. This child was one of a number of children who had played with a puppy which became ill and died several weeks before onset of the child's illness. It is interesting
to note that the diagnosis in this case was not made until post mortem and if there had been no autopsy the case would not have been diagnosed.

The second case of human rabies recorded for the United States this year was reported in an 11-year-old boy who died on October eighth in Grace, Idaho, in the southeastern corner of the State. The boy was bitten on the cheek while sleeping in the backyard three or four weeks previously. He was awakened by the bite but was not able to identify the animal. The biting animal left three tooth marks on the upper left cheek and one mark on the lower left cheek. It is believed that the biting animal may have been a bat. The boy received no treatment, was admitted to the hospital on October fifth, and died on October eighth.

**U.S. HUMAN RABIES DEATHS - 1962**

<table>
<thead>
<tr>
<th>Locality</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>Cameron County, Texas</td>
<td>7/24/62</td>
<td>4</td>
<td>M Unknown</td>
<td>Un-known</td>
<td>11 days</td>
<td>None</td>
<td>Unknown - probably dog</td>
</tr>
<tr>
<td>Caribou County, Idaho</td>
<td>10/8/62</td>
<td>11</td>
<td>M Bitten on cheek</td>
<td>3 or 4 weeks</td>
<td>4 days</td>
<td>None</td>
<td>Animal unidentified - possibly bat</td>
</tr>
</tbody>
</table>

DHEW/PHS/CDC - Atlanta, Ga., January, 1963
HUMAN RABIES DEATHS - 1962 TO DATE

At this writing (August 23, 1963) there have been no human rabies deaths for 1963.

THE RABIES PICTURE - OVER THE YEARS

Although the number of rabies cases in dogs declined, the number of wildlife cases increased during 1962. There were 257 more cases reported in 1962 than in 1961. The decline since 1946 and the leveling-off in the last three years is illustrated in the following table:

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Wildlife</th>
<th>Cats</th>
<th>Livestock</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>8,384</td>
<td>956</td>
<td>452</td>
<td>1,055</td>
<td>22</td>
<td>10,872</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>2,079</td>
<td>371</td>
<td>794</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>1,836</td>
<td>277</td>
<td>645</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>2,174</td>
<td>217</td>
<td>482</td>
<td>3</td>
<td>3,470</td>
</tr>
<tr>
<td>1962</td>
<td>565</td>
<td>2,314</td>
<td>232</td>
<td>614</td>
<td>2</td>
<td>3,727</td>
</tr>
</tbody>
</table>

UNITED STATES-CANADA BORDER REGIONAL RABIES CONFERENCE HELD

A United States-Canada Border Regional Rabies Conference was held on March 28 and 29, 1963, at the University of Vermont, Burlington. A preliminary survey had revealed an increase in animal rabies along the eastern portion of the United States-Canada border. Eastern progression of the disease from the Province of Quebec had begun to involve Vermont, New Hampshire and Maine, states that have been virtually rabies-free for the last 28 to 35 years. The purpose of the meeting was to develop the initial phase of a control program for the general eastern border area.

The meeting was sponsored by the Vermont Department of Health, the Department of Epidemiology and Community Medicine, University of Vermont College of Medicine, and the Communicable Disease Center.

Participating in the conference were representatives of all agencies interested in and responsible for the prevention and control of rabies on national, state and local levels. These included Federal, state and local health departments, United States and Canadian fish and wildlife services, state and provincial game commissions, state and national veterinary services and others.

The first day of the conference was devoted to a review of the most recent advances in laboratory diagnosis, control of dog rabies, management of human exposure, pre-exposure immunization of high-risk groups, and wildlife rabies. The following day featured a regional workshop during which concrete plans for a coordinated control program were drawn up. These plans include a monthly surveillance program for New York, Vermont, New Hampshire, Maine and Quebec, with the Boston Regional Office of the Public Health Service as the collection and distribution point.
**REPORT OF COMMITTEE**

**RABIES IN ANIMALS**

First Six Months—1962-1963

(CDC—United States Public Health Service)

<table>
<thead>
<tr>
<th>State</th>
<th>1962 (First Six Months)</th>
<th>1963 (First Six Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEW ENGLAND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Connecticut</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SOUTH ATLANTIC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Virginia</td>
<td>62</td>
<td>91</td>
</tr>
<tr>
<td>North Carolina</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>South Carolina</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Georgia</td>
<td>5</td>
<td>37</td>
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<td>Florida</td>
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<td>55</td>
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<td></td>
</tr>
<tr>
<td>Rhode Island</td>
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</tr>
<tr>
<td>Virginia</td>
<td>80</td>
<td>114</td>
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**MIDDLE ATLANTIC**

<table>
<thead>
<tr>
<th>State</th>
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<th>1963</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>New Jersey</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>EAST SOUTH CENTRAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td>Tennessee</td>
<td>153</td>
<td>73</td>
</tr>
<tr>
<td>Alabama</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Mississippi</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SOUTH ATLANTIC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Virginia</td>
<td>62</td>
<td>91</td>
</tr>
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<td>North Carolina</td>
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<tr>
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<td>7</td>
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<td>Georgia</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td>Florida</td>
<td>29</td>
<td>55</td>
</tr>
<tr>
<td>EAST NORTH CENTRAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>259</td>
<td>211</td>
</tr>
<tr>
<td>Indiana</td>
<td>149</td>
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</tr>
<tr>
<td>Illinois</td>
<td>46</td>
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<td>Michigan</td>
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<td>Wisconsin</td>
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<td>WEST NORTH CENTRAL</td>
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<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>117</td>
<td>128</td>
</tr>
<tr>
<td>Iowa</td>
<td>232</td>
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<tr>
<td>Missouri</td>
<td>95</td>
<td>89</td>
</tr>
<tr>
<td>North Dakota</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>South Dakota</td>
<td>61</td>
<td>57</td>
</tr>
<tr>
<td>Nebraska</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Kansas</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>WEST SOUTH CENTRAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td>53</td>
<td>32</td>
</tr>
<tr>
<td>Louisiana</td>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>Texas</td>
<td>355</td>
<td>316</td>
</tr>
<tr>
<td>MOUNTAIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montana</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Idaho</td>
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<td>Wyoming</td>
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<td>23</td>
</tr>
<tr>
<td>Utah</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nevada</td>
<td>0</td>
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</tr>
</tbody>
</table>
| MEXICO BORDER RABIES REVIEW - 1962

During 1962, the United States Public Health Service assigned a CDC veterinary officer to the United States-Mexico border area stationed in El Paso, Texas and another to the lower Rio Grande at Brownsville. The primary function of the office at El Paso is coordinating programs between the United States border states, and through the Pan American Sanitary Bureau, with programs in Mexico.

The beginning of the Mexican Federal Rabies Control program was the most important single action relative to border rabies control in 1962.
The principal challenge of rabies control in 1962 was the San Diego Tijuana rabies epizootic which began early in September. This outbreak accounted for all of the cases reported from that area for the year. As of December 28, there had been a total of 58 cases, 34 from San Diego County and 24 from Tijuana. Six cases per week were found in San Diego during December. There are three areas of high rabies incidence in addition to the San Diego-Tijuana area. These are the Imperial-Mexicali area which had 34 rabid dogs, the Juarez-El Paso-Dona Ana area which recorded 67 rabid dogs, and the Cameron County-Matamoros area which recorded 52 rabid dogs. Weekly morbidity reports from all three areas showed a year-long, almost even distribution of cases. There were no distinct peaks or abrupt changes in numbers of cases. This type of distribution is characteristic of enzootic disease. All of the areas involved are twin-cities of medium to large population where dog rabies is a chronic problem. There is no border city with enzootic rabies where the sister city is not involved. Thus, it is apparent that neither of the cities can control rabies alone. Coordination and cooperation are essential and all border programs are now being developed along this line.

CURRENT INVESTIGATIONS

Comparative studies by the Communicable Disease Center on various routes and regimens for pre-exposure immunoprophylaxis of high risk human groups have shown no statistically significant difference between 0.2 ml. intradermal and 1.0 ml. deep subcutaneous of duck embryo vaccine. Furthermore, there was no significant difference in the spacing of the primary series between one week and one month apart. The latest recommendations for this reduced rabies vaccine regimen is a primary series of three inoculations spaced one week apart, each consisting of 1.0 ml. duck embryo vaccine deep subcutaneously in the upper arm, followed by a booster, same dose and route, six months later.

Preliminary studies by CDC on an indirect fluorescent antibody (IFA) test for measuring rabies serum antibodies have shown promising results. Out of 588 sera tested by the IFA and SN tests, 285 were positive by the SN test and 280 positive by the IFA test, showing 98.3 percent agreement. In another series of serum samples from immunized persons, the IFA method showed a greater number of positives than the SN method, indicating that the IFA test is more sensitive than the SN test for detecting specific circulating serum antibodies.

In a comparative antigenicity test of human rabies vaccine at the CDC rabies investigations laboratory, no statistically significant difference in protection was evident in dogs between duck embryo rabies vaccine and Semple type rabies vaccine. Both groups were inoculated in a single dose with an equivalent of 10 human doses of each vaccine and challenged two months later.
REPORT OF THE COMMITTEE ON RESOLUTIONS

J. G. Milligan, Montgomery, Alabama, Chairman; W. L. Bendix, Richmond, Virginia; F. G. Buzzell, Augusta, Maine; A. P. Schneider, Boise, Idaho

Gentlemen, your Committee has discussed resolutions presented to us and recommended the following for your consideration and judgment. I hope what we have done will meet with the approval of this association. We have two resolutions presented to us by the members of the Committee on Stockyards, Markets and Transportation. They deal with the subject of Foot and Mouth Disease.

RESOLUTION NO. 1
Foot and Mouth Disease

WHEREAS, The United States has been free of Foot and Mouth Disease since 1929, and

WHEREAS, Many countries in the Western Hemisphere have become infected during the last decade, and

WHEREAS, It has been estimated that the entry and spread of Foot and Mouth Disease in the United States would cost the livestock industry in excess of 200 million dollars per year, and

WHEREAS, Reliable information indicates that Foot and Mouth Disease virus is present in Cuba, and large numbers of Cuban Refugees continuously enter the United States, thus making possible the accidental or intentional introduction of the virus into this country, and

WHEREAS, The Animal Inspection and Quarantine Division is charged with the responsibility of preventing the introduction of exotic diseases into the United States; and due to the lack of funds in recent years, this division has not been able to increase its personnel and expand its facilities to keep pace with the increased demands placed upon it by the rapid growth of international travel and commerce, not to mention the increased immediate danger of the Cuban threat,

THEREFORE BE IT RESOLVED, That the United States Livestock Sanitary Association urge Congress to appropriate adequate funds to enable the Animal Inspection and Quarantine Division to provide the degree of protection that should be given to the livestock industry which is so vital to the welfare of the nation, and

BE IT FURTHER RESOLVED, That copies of the resolution be sent to all National Livestock Organizations, the Chairman of the Senate and House Agricultural Committee, the Secretary of Agriculture, the Director of Bureau of Budgets and the Administration of the Agriculture Research Service.
RESOLUTION NO. 2
Foreign Disease Control

WHEREAS, The United States and Foreign interests are attempting to import prohibited animals or their offsprings into the United States by passage through or temporary stay in an intermediate country, and

WHEREAS, This violates the intent and spirit of existing statutes and thus constitutes a threat to this country by weakening our defense against the introduction of foreign disease,

THEREFORE BE IT RESOLVED, That the United States Livestock Sanitary Association recommend that the United States Department of Agriculture prohibit the entry of animals or their offsprings into the United States from any country if such animals originate in any country from which the direct importation of animals is prohibited.

BE IT FURTHER RESOLVED, That copies of this resolution be sent to all National Livestock Organizations, the Chairman of the Senate and House Agricultural Committees, the Secretary of Agriculture and the Administrator of the Agriculture Research Service.

RESOLUTION NO. 3
Twenty-Eight Hour Law

WHEREAS, The twenty-eight hour law, specifically known as Public Law 340, was originally designed to provide for the humane treatment of livestock in transit, and

WHEREAS, Today only nine percent of the livestock are handled by rail and are subject to Public Law 340, and

WHEREAS, Ninety-one percent of the livestock are handled by trucks or other conveyances and are not subject to Public Law 340,

THEREFORE BE IT RESOLVED, That the Congress of the United States be requested to amend Public Law 340 to include the movement of livestock in interstate commerce by truck or other conveyances,

BE IT FURTHER RESOLVED, That copies of this resolution be mailed to the Senate and House Interstate Commerce Committees, to each of the State Departments of Agriculture and State Livestock Disease Control officials, the United States Secretary of Agriculture and the American Humane Society.

RESOLUTION NO. 4
Health Certificates

WHEREAS, A proposed National Federal Interstate Health Certificate has been presented to this Association and to all of the State Livestock Sanitary officials for consideration, and
WHEREAS, We have been advised that Federal legal statutes require the use of this certificate in three specific areas of regulation, and

WHEREAS, At this time and for many years past, livestock in inter-state commerce has legally moved accompanied by individual state certification originated by the state of origin, and

WHEREAS, There still remains in the minds of many responsible State officials doubt and confusion as to both the legal status and the legal necessity of either or both State or Federal interstate certificates,

THEREFORE BE IT RESOLVED, By this Association assembled in Albuquerque, New Mexico, in October, 1963, that the United States Department of Agriculture is urgently requested to defer any further action toward the use of this proposed Federal certificate until such time as the Program and Policy Committee of the United States Livestock Sanitary Association has had the opportunity to meet with the appropriate representatives of the United States Department of Agriculture including the United States Department of Agriculture Legal Council and obtain a thorough understanding of both its legal implications and its practical applications, and said Program and Policy Committee has had time to fully inform the Executive Committee of this Association as to its conclusion.

RESOLUTION NO. 5
Ram Epididymitis

WHEREAS, Ram Epididymitis is of major importance in the United States, and

WHEREAS, The incidence of Epididymitis becomes more widespread daily, and

WHEREAS, Methods of detection and prevention are rudimentary,

BE IT HEREBY RESOLVED, That each of the several states undertake a survey of sheep flocks within their states to determine by physical examination an approximation of the incidence and percentage of infection within the states and to furnish to the Committee prior to the next annual meeting all data gathered for compilation and distribution,

BE IT FURTHER RESOLVED, That all possible sources of revenue be contacted for additional research funds to supplement those previously granted and expended by NIH.

RESOLUTION NO. 6
Special Licensing of Biologics

WHEREAS, The sixty thousand dollars of research funds expended by NIH in attempt to develop an Epididymitis vaccine, has resulted in an experimentally effective method of treatment,
THEREFORE BE IT RESOLVED, That the United States Department of Agriculture approve production of the developed R. E. O. BACTERIN, under special license, for manufacture and distribution on a limited scale, the administration and use of such treatment to be strictly regulated.

RESOLUTION NO. 7
Foot Rot of Sheep

WHEREAS, Foot Rot of sheep has long been a major problem in many sections of the United States, and

WHEREAS, This disease causes great annual economic losses, and

WHEREAS, Basic research on cause and transmission of this disease is not fully known,

THEREFORE BE IT RESOLVED, That the United States Department of Agriculture be requested to institute a research program through one of the federal laboratories and that such research program be continued and financially supported with a view toward eradication of this disease.

RESOLUTION NO. 8
Scrapie

WHEREAS, The current program of scrapie control and eradication has been apparently successful, your Committee recommends that no change be made in the National Scrapie Eradication Program at this time. We further recommend continued study during the ensuing year and be re-evaluated just prior to the next meeting of the Association. Those states not providing indemnity for Scrapie eradication or that are only providing inadequate indemnity are again urged to take necessary steps to provide adequate indemnity.

RESOLUTION NO. 9
Scabies

WHEREAS, The Committee on Parasitic Diseases of Sheep has reported on Scabies programs in the several states, and

WHEREAS, Scabies is a transmissible disease, your Committee hereby resolves that the Animal Disease Eradication Division immediately contact the several states and develop a uniform national standard for establishing Scabies Free Areas, that all states now and in the future comply with the standard once it has been established; and further that the same group immediately embark upon the development of a uniform program for the maintenance of Scabies Free Areas.
REPORT ON THE INFORMAL DISCUSSIONS ON
ANIMAL VIRUS CHARACTERIZATION

Hanover, 17 August 1963

LIST OF PARTICIPANTS

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United States of America

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Dr. G. R. Scott
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Royal (Dick Veterinary School
University of Edinburgh
Edinburgh
Scotland
1. Introduction

An informal meeting of veterinary virologists attending the XVIIth World Veterinary Congress, Hanover, was convened on 17 August in the Veterinary High School. Dr. Kaplan, in opening the meeting, described the work of WHO in the field of classification of viruses isolated from man and animals. Special reference was made to the recommendations of the WHO Informal Meeting on Comparative Virology held in New York last year which included the circulation of a questionnaire to laboratories investigating veterinary viruses in order to elicit information on the virus strains maintained by them. This questionnaire had already been circulated and the replies are being received for the United States Livestock Sanitary Association at the School of Public Health, University of California, Berkeley 4, California (Western Hemisphere) and the Virus Research Institute, Pirbright, Surrey (Eastern Hemisphere).

The meeting commended the work already done by WHO in collaboration with the International Association of Microbiology and FAO on classification of virus isolated from man and animals. The following agenda of subjects was discussed:

1) Revision of the working list of viruses isolated from diseases of domestic animals as prepared at the Informal Meeting on Comparative Virology in New York.

2) Determining priorities of economically important animal viruses and virus groups for classification and standardization of identification procedures.

3) Discussion of replies to the questionnaire received so far and to suggest follow-up action to get further information.

4) Preparation of specific reference sera against viruses which are well defined and suitable for this purpose.
5) Other aspects of work on virus classification including meetings, publications and progress reports.

2. Revision of list of viruses

In revising the list already prepared at the Informal Meeting on Comparative Virology in New York the Group felt that all viruses of animals, with the exception of those of man, primates, and viruses of the arbovirus group, should be considered in the cataloguing effort. Priority consideration would be given to characterizing viruses of the domesticated animals. The arbovirus group is being handled by the Virus Unit in WHO in collaboration with the American Committee on Arthropod-borne Viruses.

The characteristics of the following viruses were considered to be sufficiently well-known to warrant their being taken up as a first group for inclusion in the system of classification:

<table>
<thead>
<tr>
<th>Name of virus</th>
<th>Provisional viral group to which the virus may belong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian infectious bronchitis</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Avian infectious laryngotracheitis</td>
<td>Herpes</td>
</tr>
<tr>
<td>Canine distemper</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Contagious ecthyma (&quot;Orf&quot;)</td>
<td>Poxvirus</td>
</tr>
<tr>
<td>Duck influenza (type A)</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Equine influenza (type A)</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Equine rhinopneumonitis (equine virus abortion)</td>
<td>Herpes</td>
</tr>
<tr>
<td>Equine and bovine rhinoviruses</td>
<td>Picornavirus</td>
</tr>
<tr>
<td>Feline infectious enteritis (feline panleukopenia)</td>
<td>Picornavirus</td>
</tr>
<tr>
<td>Foot and mouth disease</td>
<td>Picornavirus</td>
</tr>
<tr>
<td>Fowl plague (type A influenza)</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Infectious canine hepatitis</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>Infectious fibromas-infectious myxomatosis of rabbits</td>
<td>Poxvirus</td>
</tr>
<tr>
<td>Infectious papillomatosis (wart viruses)</td>
<td>Papova</td>
</tr>
<tr>
<td>Newcastle disease</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Parainfluenza viruses</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Pseudorabies (Aujeszyk's disease)</td>
<td>Herpes</td>
</tr>
<tr>
<td>Rabies</td>
<td>Myxovirus</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>Myxovirus</td>
</tr>
</tbody>
</table>
Name of virus | Provisional viral group to which the virus may belong
--- | ---
Rous sarcoma | Myxovirus
Swine influenza | Myxovirus
Vesicular exanthema | Picornavirus

The following viruses might be ready for including in this list in the near future:

Hog cholera (swine fever)

Infectious bovine rhinotracheitis (infectious pustular vulvo-vaginitis) | Herpes

Infectious porcine encephalomyelitis (Teschen Disease, Talfan Disease) | Picornavirus

Vesicular stomatitis

3. Designation of priority

The Group proposed the following viruses to be taken up as first priority for designation of reference strains and preparation of reference sera:

1. Rinderpest
2. Vesicular exanthema
3. Infectious bovine rhinotracheitis
4. Virus diarrhoea of cattle
5. Swine enteroviruses
6. Influenza A group viruses of animals including equine influenza, duck influenza, swine influenza, fowl plague
7. Newcastle disease
8. Parainfluenza III
9. Canine distemper
10. Canine hepatitis

4. Discussion of replies received

The replies to the questionnaire already circulated were outlined in a document prepared by Dr. Brooksby, but as a large number of replies were still expected, the Group did not attempt to review the information received. However, a few difficulties in obtaining the desired information were discussed and a revision of certain questions was proposed. It was agreed that provision should be made for receiving information on virus isolates on which only incomplete data was available. A group was appointed to help rectify this difficulty by re-drafting the questionnaire.
5. Other aspects of virus classification work

In reviewing the revised list of animal viruses, it was observed that many of the viruses fell in two groups, that is, Myxovirus and Picornavirus, although other groups such as Herpes, Poxvirus and Papovavirus were also included. The Group recommended that a representative from the western and eastern committees (see below) be appointed on the existing Human Respiratory and Enterovirus Committees.

In the Western hemisphere a committee of the United States Livestock Sanitary Association is already dealing with the work of animal virus classification. No such committee, however, exists in the eastern hemisphere where national groups and even individual laboratories are working without opportunities for close collaboration. It was proposed that the following virologists should constitute a committee of the eastern hemisphere:

Dr. A. O. Betts, School of Veterinary Medicine, Department of Animal Pathology, University of Cambridge, Madingley Road, Cambridge, United Kingdom

Dr. K. Bögel, Federal Research Institute for Animal Virus Diseases, Wilhelmstrasse 82, Tübingen 74, Germany

Dr. J. B. Brooksby, Research Institute, Pirbright, Surrey, United Kingdom

Dr. F. Bürki, Veterinary-Bacteriological Institute, University of Berne, Engehaltenstrasse 6, Berne, Switzerland

Professor V. Cilli, Instituto Malattie Infettive, Facolta Medicina Veterinaria, Via S. Costanzo 4, Perugia, Italy

Dr. Z. Dinter, Institute of Virology, University of Uppsala, Uppsala, Sweden

Dr. E. French, Animal Health Research Laboratory, Division of Animal Health, C.S.I.R.O., Private Bag No. 1, Parkville, N.2, Victoria, Australia

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Dr. T. Omori, National Institute of Animal Health, Kodaira-Machi, Kitatsama, Tokyo, Japan

Dr. A. Paraf, Ecole Nationale Vétérinaire, Alfort (Seine), France

Dr. A. B. Paterson, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, United Kingdom

Professor F. Patůcka, Head, Department of Microbiology, Faculty of Medicine, Charles University, Prague, Czechoslovakia

Dr. W. Plowright, East African Veterinary Research Organization, Mugua, P.O. Box 32, Kikuyu, Kenya, East Africa
As the next International Congress of Microbiology, to be held in 1966, will take up virus classification as one of the items for discussion, the two Committees would prepare a publication or a progress report for discussion before that date.

**REVISED LIST OF RELATIVELY WELL-DEFINED VIRAL ISOLATES* AND DISEASES OF DOMESTICATED ANIMALS**

- Adenoviruses (orphan) Gal virus, cattle
- African horse sickness
- African swine fever
- Avian encephalomyelitis (epidemic tremor)
- Avian infectious bronchitis
- Avian infectious laryngotracheitis
- Blue tongue
- Borna disease
- Bovine papular stomatitis
- Bovine rhinovirus
- Canine distemper
- Contagious ecthyma ("Orf")
- Duck influenza (type A)
- Duck virus hepatitis
- Encephalomyocarditis
- Equine artherosclerosis
- Equine encephalomyelitis - Eastern, Venezuelan, Western
- Equine infectious anaemia
- Equine influenza (types A)
- Equine rhinopneumonitis (equine virus abortion)
- Equine rhinovirus
- Feline infectious enteritis (feline panleukopenia)
- Feline viral rhinotracheitis
- Foot and mouth disease
- Fowl leukemia (different types)
- Fowl plague (type A influenza)
- Hog cholera (swine fever)
- Infectious bovine rhinotracheitis
  (infectious pustular vulvo-vaginitis)
- Infectious canine hepatitis
- Infectious fibromas of rabbits
- Infectious papillomatosis (wart viruses)
- Infectious porcine encephalomyelitis
  (Talsen Diseases, Talfan Disease)
- Japanese B encephalitis
- Louping ill
- Lumpy skin disease
- Lymphocytic choriomeningitis
- Malignant catarrhal fever
- Mucosal disease - virus diarrhoea
- Myxomatosis
- Nairobi disease of sheep
- Newcastle disease
- Parainfluenza viruses
- Picornaviruses (orphan) - bovine, Feline, swine and other
- Pox viruses - cow, fowl, goat, horse, rabbit, sheep and swine
- Pseudocowpox (milker's nodules)
- Pseudorabies (Aujeszky's disease)
- Rabies
- Rift valley fever
- Rinderpest
- Rous sarcoma
- Swine influenza
- Tick-borne encephalitis
- Transmissible gastro-enteritis of piglets
- Turkey encephalitis
- Vesicular exanthema
- Vesicular stomatitis
- Wesselsbron disease

*Rickettsial and Psittacosis LGV isolates are not included.
FACTORS THAT INFLUENCE ANTIBODY RESPONSE

Ben Sheffy, Ph.D. and John Gilmartin, M.S.

Ithaca, New York

Considerable variation has been observed in the response to immunization procedures of animal populations under field conditions. Although the effects of genetics are recognized upon the immune response, certain treatments and altered physiological conditions within the host also have been shown to influence acquired immunity. Acquired immunity is the result of cellular production of antibody which proceeds in two phases, primary response, or the induction phase, wherein antigen prepares cells to produce antibody, and secondary response, or the production phase, during which actual production of antibody occurs. Because antibody can be measured, two determinations can be made: (1) the time required for formation of antibody after introduction of antigenic stimulus and (2) the amount of antibody that is formed.

In making measurements, therefore, experimental conditions of test must be controlled, and, in order to avoid error, variables must be controlled for significant results. The type of antigen used is an important factor. A particular living antigen that is capable of multiplying will cause antibodies to appear within a certain period of time in a genetically similar population, if there has been no treatment or altered physiology. The time interval to form antibodies may vary with another living antigen and certainly will vary if the antigen has been inactivated. An inactivated antigen inoculated once induces primary response with little or no measurable antibody unless mixed with an adjuvant. A second inoculation of the inactivated antigen causes secondary response characterized by accelerated

<table>
<thead>
<tr>
<th>Table I</th>
<th>Effect of Type of Antigen on Immune Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>Homotyptic</td>
<td></td>
</tr>
<tr>
<td>Live Single Inc.</td>
<td>+</td>
</tr>
<tr>
<td>Repeated Inc.</td>
<td>-</td>
</tr>
<tr>
<td>Inactivated Single Inc.</td>
<td>+</td>
</tr>
<tr>
<td>Repeated Inc.</td>
<td>-</td>
</tr>
<tr>
<td>Inactivated Single Inc. + Adjuvant</td>
<td>+</td>
</tr>
<tr>
<td>Repeated - Adjuvant</td>
<td>-</td>
</tr>
<tr>
<td>Heterotypic</td>
<td></td>
</tr>
<tr>
<td>Single Inc.</td>
<td>+</td>
</tr>
<tr>
<td>Repeated Homotypic</td>
<td>-</td>
</tr>
</tbody>
</table>

From the Veterinary Virus Research Institute, Cornell University, Ithaca, New York, and Eaton Laboratories, Norwich, New York. Supported by a grant from the Office of Naval Research.
FACTORS THAT INFLUENCE ANTIBODY RESPONSE

TABLE II
Effects of Various Treatments on Immune Response

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Immune Response Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Primary</td>
</tr>
<tr>
<td>Cortisone (1–2)</td>
<td>+</td>
</tr>
<tr>
<td>Irradiation (3–6) sublethal lethal</td>
<td>+</td>
</tr>
<tr>
<td>Hypophysectomy (4)</td>
<td>–</td>
</tr>
<tr>
<td>Adrenalectomy (5)</td>
<td>–</td>
</tr>
<tr>
<td>Thyroidectomy (5)</td>
<td>+</td>
</tr>
<tr>
<td>Thymectomy (7)</td>
<td>+</td>
</tr>
<tr>
<td>Central Nervous System (4)</td>
<td>Not determined</td>
</tr>
<tr>
<td>Conditioned stimulus</td>
<td></td>
</tr>
<tr>
<td>Unconditioned stimulus</td>
<td></td>
</tr>
<tr>
<td>Antimetabolites</td>
<td></td>
</tr>
<tr>
<td>6 Mercaptopurine (8)</td>
<td>+</td>
</tr>
<tr>
<td>5 Bromouracil deoxyriboside (9)</td>
<td></td>
</tr>
<tr>
<td>B3-thienyl alanine (10)</td>
<td>+</td>
</tr>
<tr>
<td>Deoxypyridoxine (11)</td>
<td>+</td>
</tr>
<tr>
<td>A-methopterin (12)</td>
<td>+</td>
</tr>
<tr>
<td>Methotrexate (13)</td>
<td>+</td>
</tr>
<tr>
<td>Antimicrobial Agents</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>–</td>
</tr>
<tr>
<td>Furoxone</td>
<td>–</td>
</tr>
</tbody>
</table>

+ means effect on antibody production; –, no effect.

antibody formation. A new concept, termed heterotypic, has been described, which indicates that inoculation of a member of organisms that are group related induces primary response to other members of the same group in a manner similar to a single inoculation of inactivated antigen.14

Selection of antigen, therefore, can be a controllable variable, and in a genetically similar population, a treatment or altered physiology can be evaluated. In Table II, present knowledge has been summarized. It should be noted that primary response or secondary response or both may be affected.

Depression of antibody by cortisone has been related directly to diminution of lymphoid tissue.1,2 When cortisone treatment was started at least two days before antigen was given and continued, primary response was affected. A similar result has been reported for effects of irradiation.3 Primary response in rabbits was inhibited completely by whole body x-radiation of 400r given 12–48 hours prior to but not after inoculation of antigen, whereas 800r had only a minimal effect on secondary response. Although greater depression of secondary response has been reported,6 preformed antibody was unaltered and its natural rate of decline was unchanged. Studies aimed at determination of the role of endocrine glands in
regulation of antibody production indicated that hypophysectomy or adrenalectomy in rats did not significantly affect antibody production.\textsuperscript{4} Thyroidectomy,\textsuperscript{4} however, resulted in diminished antibody production. Administration of thyroxine shortened the period between administration of antigen and appearance of antibody. Complete thymectomy totally suppressed primary response.\textsuperscript{7} The role of the central nervous system in immunological responses has not been studied extensively. Specific nutrient antagonists have been shown to inhibit the immune response.

Antibody production apparently can be influenced by various treatments and by altered physiology. Nutrition of the host also would be expected to influence antibody formation. Antibodies are protein and must be synthesized within host cells. Therefore, not only must essential amino acids be available but other nutrients that affect synthesis of antibodies must be available. Current information has been reviewed and this has been summarized in Table III, where it can be seen that severe protein deficiency, specifically amino acids, and deficiency of certain B-vitamins have been shown to depress antibody production markedly, while vitamins A, D, and E did not.

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrients Which Affect Antibody Production</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibody Production Impairment</th>
<th>Marked</th>
<th>Slight</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophane</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-Vitamins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bivoflavin</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic Acid</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chollne</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Fat Soluble Vitamins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>K - not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals - Not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids - Not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates - Not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effects of panothenic acid and folic acid on antibody response of dogs given live distemper and infectious canine hepatitis vaccine were studied. Results are presented here.
FACTORS THAT INFLUENCE ANTIBODY RESPONSE

MATERIALS AND METHODS

From our disease-free kennel, pure-bred Beagle dogs four to five weeks of age were placed in individual metabolism cages in isolation units and then were fed a purified diet (Table IV) deficient in either pantothenic acid or folic acid. After an appropriate depletion period, each dog was fed daily a measured quantity of either calcium pantothenate or folic acid that was 0.5, one, or a multiple of the requirements recommended by the National Research Council (N.R.C.). At the time supplementation began, a single inoculation of a combination attenuated vaccine of distemper virus and infectious canine hepatitis virus was given subcutaneously. Serum from each dog was collected at the time of vaccination and at various intervals of time afterwards. Neutralization tests for presence and amount of antibody were made and the results are presented in Tables V and VI.

TABLE IV
Purified Dog Diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (Vitamin Free)</td>
<td>20.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30.0</td>
</tr>
<tr>
<td>Cerelose</td>
<td>34.0</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>9.5</td>
</tr>
<tr>
<td>Cod Liver Oil</td>
<td>0.5</td>
</tr>
<tr>
<td>Salts IV*</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin Mix**</td>
<td>1.0</td>
</tr>
<tr>
<td>Sulfasuxidine</td>
<td>1.0</td>
</tr>
</tbody>
</table>


**The vitamin mixture supplied 1.5 mg thiamine, 4.0 mg riboflavin, 2.0 mg pyridoxine, 20.0 mg niacin, 20.0 mg menadione, 0.05 mg biotin, 0.05 mg vitamin B12, 20.0 mg alpha tocopherol, 25.0 mg inositol, and 900.0 mg chlorine chloride per pound of diet.

TABLE V
Antibody Response of Dogs to Vaccination Fed Different Levels of Calcium Pantothenate

<table>
<thead>
<tr>
<th>No. Dogs</th>
<th>Supplement*</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
</tr>
<tr>
<td>2</td>
<td>10x</td>
<td>0</td>
<td>0.8/0.92</td>
<td>2.3/2.8</td>
<td>2.7/3.2</td>
</tr>
<tr>
<td>2</td>
<td>5x</td>
<td>0</td>
<td>0.3/0</td>
<td>2.3/2.7</td>
<td>2.7/3.1</td>
</tr>
<tr>
<td>2</td>
<td>2x</td>
<td>0</td>
<td>0/0</td>
<td>2.7/2.7</td>
<td>2.6/3.0</td>
</tr>
<tr>
<td>1</td>
<td>1x</td>
<td>0</td>
<td>0</td>
<td>1.9/2.5</td>
<td>2.6/2.5</td>
</tr>
<tr>
<td>2</td>
<td>0.5x</td>
<td>0</td>
<td>0***</td>
<td>1.6/1.5***</td>
<td>-- --</td>
</tr>
</tbody>
</table>

*x = .045 mg calcium pantothenate per pound body weight per day.

**ICH is abbreviation for infectious canine hepatitis, CD for canine distemper.

***Died
In addition, white cell counts and hemoglobin determinations were made weekly by standard hematologic techniques. Body weights were recorded twice each week.

RESULTS

Pantothenic Acid: After one week of depletion, several dogs developed an erratic appetite, and these dogs showed slight decreases in weight gains during the second week of depletion. No changes in hemoglobin or leukocyte counts were observed. Growth rate after supplementation was related to the level of calcium pantothenate given. Dogs which received no supplement, or 0.5 times the N.R.C. recommended allowance, died between the third and sixth week of test, after sudden prostration and coma. No significant differences in the average daily gain were noted in those dogs supplemented with two, five, or 10 times the N.R.C. recommendations. The gains of these dogs were 54, 46, and 53 grams respectively. However, the dogs supplemented with five and 10 times the N.R.C. recommendations produced higher and earlier antibody titers. Daily dietary requirements of young dogs for calcium pantothenate, therefore, appeared to be between 45 and 90 micrograms for each pound of body weight, if growth was considered the criterion of sufficiency, although higher levels were required for optimum immune response.

Folic Acid: After nine weeks of depletion, all dogs developed erratic appetites and weight gains decreased. Total white blood cell counts decreased slightly but remained in the normal range. No changes in hemoglobin levels were recorded. After supplementation, dogs grew normally. In depleted dogs, severe impairment of antibody production against both distemper and infectious hepatitis antigens were noted (Table VI). Whereas antibodies were found in supplemented dogs eight days after vaccination, those depleted required a period of 17 to 21 days before antibodies were detected. Daily requirements of young dogs for folic acid were found to be less than six micrograms for each pound of body weight.

TABLE VI
Antibody Response to Vaccination of Dogs Fed Different Levels of Folic Acid

<table>
<thead>
<tr>
<th>Supplement</th>
<th>0</th>
<th>8</th>
<th>13</th>
<th>17</th>
<th>21</th>
<th>27</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
<td>ICH CD</td>
</tr>
<tr>
<td>20x</td>
<td>*</td>
<td>1.3/0.92</td>
<td>2.7/2.3</td>
<td>2.7/2.8</td>
<td>2.7/2.8</td>
<td>2.7/2.8</td>
<td>2.7/2.8</td>
</tr>
<tr>
<td>2x</td>
<td>*</td>
<td>0.3/1.3</td>
<td>2.7/2.8</td>
<td>2.7/3.1</td>
<td>2.7/3.1</td>
<td>2.7/3.5</td>
<td>2.7/3.5</td>
</tr>
<tr>
<td>1x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/0.9</td>
<td>0/0.9*</td>
<td>2.7/3.0</td>
<td>3.4/2.9</td>
</tr>
<tr>
<td>0.5x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3/0</td>
<td>0.3/0.8*</td>
<td>2.2/2.1</td>
<td>2.7/2.3</td>
</tr>
</tbody>
</table>

*Supplementation began. x = 12.5 mcg Folic acid per pound body weight per day.
**ICH is abbreviation for infectious canine hepatitis, CD for canine distemper.
CONCLUSIONS

In dogs dietary deficiencies of pantothenic acid or of folic acid markedly affected but did not completely suppress antibody production after distemper and infectious canine hepatitis vaccination. The period between vaccination and appearance of neutralizing antibodies was longer and early antibody levels were lower.

REFERENCES

INTRODUCTION

Veterinary biologics are one of the elements which contribute to a safe, wholesome, and economical food supply for the nation. There are many factors which sustain this important part of our standard of living. Likewise, there are many contributors in providing safe, potent, and economical veterinary biologics. Research institutions, the biologics industry, and the Federal regulatory agency join forces in this common endeavor.

Federal requirements, licensing, and inspection activities have been described recently by Peacock and by Van Houweling. Authority to prohibit and prevent interstate sale or distribution of any veterinary biologics which are worthless, contaminated, dangerous, or harmful is contained in the Virus-Serum-Toxin Act approved March 4, 1913. The Animal Inspection and Quarantine Division (AIQD) of the Agricultural Research Service (ARS) is the responsible Federal regulatory agency for veterinary biologics.

New facilities to provide laboratory support for programs of AIQD which relate to veterinary biologics have also been described by Van Houweling and by Oshel. All functions of this laboratory support are related to a program of AIQD whereas AIQD is concerned not only with biologics but with other major programs as well.

The scope of activities and methods used by AIQD for biologics was static for many years. The range of products during that period was also relatively unchanging so that the original methods for licensing and inspection of production for hog-cholera virus and anti-hog-cholera serum were adequate.

Licensing is still based primarily on documentation by applicants of methods of production and testing, results of laboratory and field testing, production and testing facilities, personnel qualifications and research data.

Inspection has been expanded to include other products in addition to detailed inspection of every production and control process in the preparation of hog-cholera virus and anti-hog-cholera serum. This added inspection is a spot-check system used to verify that these other products are produced, tested, and labeled in accordance with the licensing documents.

*Chief, Biologics Services, National Animal Disease Laboratory.

**Assistant Director, Regulatory, National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.
Practically all veterinary biologics in the United States are produced by licensed commercial manufacturers. At the present time there are approximately 64 companies producing over 100 different products, forms, and combinations with a gross annual product estimated to value about 81 million dollars. There are over 50 animal diseases or agents for which biologics are produced.

Chart 1 represents the relationship between products used for the various diseases expressed in descending order in terms of percent of dollars of gross annual production for 1960, 61, and 62, and total dollars for each of the three years. The total has increased nearly 20 million dollars during this period.

Chart 2 is a graphic expression of the same information including also the current status of standard requirements for testing. Usually this comparison is made in terms of numbers of doses. Although the method here adds the variable of pricing, it is a common denominator and less of a variable than the wide differences in dosage.

Products for fifteen diseases each of which make up one percent or more of the total account for almost 90 percent of the biologics produced. Hog cholera biologics are one-third of this total. Of these fifteen diseases, eight are large animals, four are poultry, and three are small animal. Standard requirements for testing are established for some products in almost every one of the major disease categories.

Standard Requirements are official publications of AIQD contained in Biological Products Memo No. 60-2. They consist of a written description of testing and minimum results required by AIQD.

Facilities for Biologics Services at the National Animal Disease Laboratory (NADL) became totally operational in January, 1962, when animal quarters became available. The repository for biologics samples had been activated six months earlier so that samples of every biologic produced (except hog-cholera products) were available for assay.

The organization of Biologics Services is based on a classification of products according to host species of the antigen contained in each product. Because most diseases for which biologics are used are of greatest importance in a certain animal species or limited to a single species, this basis for organization also achieves maximum disease specialization within units. The work is divided between four product disciplines—two for large animal biologics including one exclusively for anaerobic and anthrax products, one for small animal biologics, and one for poultry biologics.

Present personnel and assigned facilities are not expected to provide continuous, concurrent surveillance of all products or immediate study of all problems relating to quality of methods. The three laboratory modules designed for this work total 7500 square feet and are divided into three
## CHART 1

Veterinary Biologics Gross Annual Production

<table>
<thead>
<tr>
<th>Disease</th>
<th>1960</th>
<th>1961</th>
<th>1962</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erysipelas</td>
<td>8.70</td>
<td>10.46</td>
<td>9.18</td>
<td>7,484,187.15</td>
</tr>
<tr>
<td>Newcastle Disease</td>
<td>8.33</td>
<td>9.15</td>
<td>7.65</td>
<td>6,240,023.57</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>5.40</td>
<td>5.50</td>
<td>5.27</td>
<td>4,295,617.65</td>
</tr>
<tr>
<td>Canine Distemper</td>
<td>5.39</td>
<td>7.19</td>
<td>5.14</td>
<td>4,213,997.07</td>
</tr>
<tr>
<td>Rabies</td>
<td>5.05</td>
<td>4.26</td>
<td>5.00</td>
<td>4,083,757.75</td>
</tr>
<tr>
<td>Shipping Fever</td>
<td>3.07</td>
<td>4.09</td>
<td>3.83</td>
<td>3,122,925.49</td>
</tr>
<tr>
<td>Fowl Cholera</td>
<td>1.73</td>
<td>3.23</td>
<td>3.53</td>
<td>2,881,109.30</td>
</tr>
<tr>
<td>Backleg</td>
<td>5.37</td>
<td>4.18</td>
<td>3.48</td>
<td>2,838,117.67</td>
</tr>
<tr>
<td>Infectious Bronchitis</td>
<td>5.15</td>
<td>4.74</td>
<td>3.46</td>
<td>2,818,657.62</td>
</tr>
<tr>
<td>Infectious Canine Hepatitis</td>
<td>2.98</td>
<td>3.64</td>
<td>3.14</td>
<td>2,562,465.48</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>2.41</td>
<td>2.59</td>
<td>3.03</td>
<td>2,472,111.24</td>
</tr>
<tr>
<td>I.B.R.</td>
<td>1.25</td>
<td>2.91</td>
<td>2.76</td>
<td>2,252,385.52</td>
</tr>
<tr>
<td>Fowl Pox</td>
<td>2.10</td>
<td>2.13</td>
<td>2.64</td>
<td>2,149,194.89</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.11</td>
<td>.60</td>
<td>1.32</td>
<td>1,074,664.50</td>
</tr>
<tr>
<td>Equine Encephalomyelitis</td>
<td>1.28</td>
<td>.99</td>
<td>0.90</td>
<td>736,821.66</td>
</tr>
<tr>
<td>Anthrax</td>
<td>1.38</td>
<td>1.01</td>
<td>.77</td>
<td>627,691.06</td>
</tr>
<tr>
<td>Fowl Laryngotracheitis</td>
<td>3.12</td>
<td>2.47</td>
<td>.74</td>
<td>605,863.60</td>
</tr>
<tr>
<td>All Other</td>
<td>10.34</td>
<td>4.64</td>
<td>8.31</td>
<td>6,750,553.79</td>
</tr>
<tr>
<td>Total</td>
<td>62,595,000</td>
<td>73,795,395</td>
<td>81,548,606</td>
<td>Up</td>
</tr>
</tbody>
</table>

## CHART 2

VETERINARY BIOLOGICAL PRODUCTS

1962

Estimated Dollar Value

$81,548,606
service areas and 25 laboratory rooms of varying size. Animal quarters and sample repository, separate from laboratory space, total 3000 square feet and are divided into 15 animal isolation and support rooms. Surveillance of products for potency, purity, or safety and studies of methods are scheduled on a priority basis.

The general objective of the biologics laboratory is to provide service needed by AIQD in connection with responsibilities under the Virus-Serum-Toxin Act. Specific objectives are: (1) assay of commercially produced animal biologics for potency, purity, and safety; (2) evaluation and improvement of existing requirements and standards; and (3) development of new requirements and standards. Based on the quality control principle, these specific objectives involve two basic areas: (1) that concerned with quality of methods of assay for potency, purity, and safety; (2) that concerned with degree of skill and integrity in application of methods by licensed laboratories. In each of these areas, the relationship is that of providing a firm basis for recommended decisions and support of these decisions.

METHODS AND RESULTS - GENERAL

Chart 3 represents the relationship between products used for the various diseases expressed graphically according to numbers of containing lots produced. Because testing is based on sampling of lots or batches,
the number of lots is indicative of possible workload. Sixty-two percent of the number of lots produced are for large animals, and more than one-third of these are anaerobic products. Fourteen percent of the number of lots produced are for small animals, and nearly an equal number are for poultry.

In the final analysis quality control depends on testing of final product and is generally recognized and accepted. Different types of products and products for different diseases vary widely in levels of potency, safety, and characteristics adaptable to practical quality control. If it is not possible to demonstrate efficacy for a product, then it follows that a practical quality measurement of potency of every production lot is impossible. There are few major products in this category. Therefore, the principle factors which control minimum level are the quality of the method used for assay of every production lot and the skill and integrity with which the method is applied.

However, in judging results as shown here (Chart 4) it is very important that the many factors which apply to quality of methods be considered in arriving at a true value for the results obtained.

Recommendations for regulatory action by Biologics Services as a result of unsatisfactory findings have been both specific, involving one or few production lots of one or more laboratories, and general, involving many lots of a few laboratories. Specific actions are those which reject or prohibit further marketing of the unsatisfactory lots. Usually these are recalled from the market. General actions are those which prohibit
further marketing of all lots of the specific product of a laboratory until corrections are made and confirmed by further testing.

METHODS AND RESULTS - SPECIFIC

Although the seriousness of some of the deficiencies revealed by testing cannot be minimized, isolated occurrence of product failure should not be cause for condemnation of the entire industry or loss of confidence in products. It cannot be expected that product quality will be the same for all lots produced or for all manufacturers. Improved methods of testing and the increased surveillance now possible does add assurance that minimum levels required are maintained.

Total surveillance of products used in national eradication programs by complete assay of every production lot prior to release or acceptance on contract purchase (Chart 5) has been an integral part of these programs for many years. This testing is now conducted by Biologics Services for Tuberculin, Pullorum Antigens, and Brucella Abortus Vaccine.

All lots of Tuberculin for contract purchase by the Animal Disease Eradication Division (ADE) are examined prior to acceptance. Forty-three percent of the total number of lots received in the sample repository during the last fiscal year were for contract. Chemical determinations include total nitrogen, nitrogen of active principle, and phenol content. Biological examinations are made for sterility, non-viable acid-fast contamination, and sensitivity. Sensitivity is determined using guinea pigs by titrating the intradermal response in sensitized and control animals for the product under test and comparing the results to that obtained for an

CHART 5

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>SAMPLES RECEIVED</th>
<th>LOTS EXAMINED</th>
<th>LOTS REJECTED</th>
<th>REJECTING FACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBERCULIN</td>
<td>72</td>
<td>31</td>
<td>14</td>
<td>1. Low Active Prin. Content</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Hypersensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. Acid Fast Contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4. Gross Contamination</td>
</tr>
<tr>
<td>PULLORUM ANTIGENS</td>
<td>150</td>
<td>150</td>
<td>11</td>
<td>1. High Density</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Hypersensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. Low Density</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4. Hypersensitivity</td>
</tr>
<tr>
<td>BRUCELLA ABORTUS VACCINE</td>
<td>1329</td>
<td>1329</td>
<td>27</td>
<td>1. Contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Low Viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. Dissociation</td>
</tr>
</tbody>
</table>
arbitrary tuberculin standard tested concurrently on the same animals. The limits of variability allowed are 90-110 percent. Forty-five percent of lots of tuberculin offered for contract purchase were rejected. Chart 5 lists rejecting factors in decreasing order of frequency of occurrence.

*Pullorum antigens* are examined for bacterial density, preservative content, and bird sensitivity. Sensitivity is determined by conducting the standard test, depending on type of product, using serum or whole blood from sensitized birds. Although a reference standard is not being used at present, several lots of antigen are examined at the same time and each is considered as a control for the group. The rejection rate for fiscal year 1963 was approximately seven percent.

Methods of examination for *Brucella Abortus Vaccine* have been standard for a long time. Each lot is checked for purity, viability, and dissociation, if any. The requirement for 10 billion organisms/cc at release is usually exceeded by several billion.

Modified live virus *Rabies Vaccine* is another product which had been under surveillance prior to operations at NADL. This early testing, conducted at Michigan State University, is continuing at NADL, including also the inactivated, tissue origin vaccines for Rabies (see Chart 6). The methods used for potency testing the two types of vaccine are different. Modified live virus vaccines are examined for virus content and animal protection. Virus content is determined by titration using tenfold dilutions of product and intracerebral mouse inoculation. Virus content required throughout the dating period is no less than $10^{3.3} \text{LD}_{50} / .03$ cc. Animal protection required is 70 percent using guinea pigs in a vaccination and challenge procedure. Inactivated vaccines are tested by a titration procedure which demonstrates difference in protection between vaccinated and unvaccinated mice. This difference is expressed in terms of protective index which must be no less than 1000. Eighteen percent of lot samples received during the first 18 months of operation at NADL were tested and 14 percent of these found to
be unsatisfactory. General regulatory action was recommended once for each type of product.

Some of the first new work started when facilities for Biologics Services became operational was examination of stock cultures used for production of certain anaerobic bacterins (see Chart 7). The principle determinations were for purity and identity starting with Clostridium hemolyticum cultures. Because some of these were found to be improperly identified or contaminated, examination of all anaerobic stock cultures was designated as high priority. Medias, methods, and biochemical reactions commonly employed in anaerobic bacteriology were used. Anaerobiasis was produced by use of a specific hydrogen for oxygen replacement in Brewer jars. Incubation for biochemical differentiation was never less than seven to 10 days. Known control cultures were included in each group of unknown cultures examined. Over 20 percent of these anaerobic stock cultures were misidentified, aerobically or anaerobically contaminated, poorly toxigenic, or failed to grow sufficiently to permit isolation and identification. General regulatory actions were necessary for one or more anaerobic product of six laboratories.

Following examination of Clostridium chauvei stock cultures, Blackleg Bacterin and combination bacterins were examined for potency by animal protection tests. The method consists of vaccination and challenge of guineas pigs and requires a minimum of 70 percent protection. Lots known to have been produced from unsatisfactory stock cultures and others were included in the testing schedule. Forty-seven percent of lots tested during this period were unsatisfactory. Correlation with the results of stock culture examination was clearly evident.

**CHART 7**
Surveillance of other major products was started as staff and equipment became available. The intent is to screen in representative numbers on all major products of all producers with emphasis on efficacy. Both products and methods are judged concurrently. Subsequent priorities and direction is charted according to the data accumulated from the screening and results of regulatory actions.

Virus content is the principle screening determination conducted for Bovine Rhinotracheitis Vaccines, Bronchitis Vaccines, and Newcastle Disease Vaccines. Animal protection tests are used for Leptospira Pomona Bacterins and Erysipelas Bacterins.

Virus content of Bovine Rhinotracheitis Vaccine is determined by using dilutions of the vaccine inoculated into cell cultures (see Chart 8). Endpoints of cytopathology are determined and virus content calculated. Satisfactory vaccine shall contain sufficient virus to yield at least $10^{4.2}\text{TCID}_{50}$ per bovine dose throughout the dating period. An extreme example of production and control failure was encountered involving this product. Regulatory actions in chronological sequence were required due to low virus content, gross contamination, virus inactivation by bacterin used as diluent, and then erroneous identity of bacterin diluent.

The results of screening of Newcastle Disease Vaccines for virus content are shown in Chart 9. After inoculation of chick embryos with tenfold dilutions of the product, endpoints of virus activity are determined by embryo death and stunting. Very few Newcastle Disease Vaccines have
failed to meet the requirement of $10^{5.0}\text{EID}_{50}/\text{ml.}$, and no general regulatory actions have been necessary on this basis. Results of limited numbers of recent bird protection tests now being conducted have correlated with results for virus content when intranasal administration is used. The correlation is less consistent for drinking water administration.

The standard of virus content for *Bronchitis vaccines* is at least $10^{3.0}\text{EID}_{50}/\text{ml.}$ (see Chart 10). Only .04 percent of those tested failed to meet the requirement. In determining virus content of combination vaccines, specific anti-NCD serum of turkey origin is used for neutralization of the Newcastle fraction.

The results for *Leptospira Pomona Bacterins* (Chart 11) appear to be equally good although one general regulatory action was recommended for this product. The method employed is an animal protection test using hamsters in a vaccination and challenge procedure. Eighty percent protection is required when 1/200 of the bovine dose is used for vaccination and the challenge dose is one log greater for vaccinates than for controls. A limited number of immunity titrations using dosages less than 1/200 of the bovine dose were satisfactory for many of these bacterins. Any failures at 1/200 of the bovine dose is cause to suspect the true identity of stock cultures used for production. Regarding the general regulatory action taken, further investigation confirmed that the suspect lot had been produced from *Leptospira canicola* serotype rather than *L. pomona*.
More than one-third of the animal space allotted to Biologics Services at NADL is used for potency screening of products for *Erysipelas* and study of potency testing methods for this group of products. Animal protection tests for *Erysipelas Bacterins* (see Chart 12) utilize both mice and pigs. Mice are used in a vaccination and challenge procedure whereby the immune response is titrated and compared to a standard product. The product under test must protect no fewer than six mice less than the standard. Pig protection is judged by comparing temperature response, symptoms, and deaths between vaccinated and control animals after intramuscular challenge. Correlation between mouse and pig potency is not always consistent. Therefore, added validity is obtained by confirmation of questionable mouse potency using the pig test. Also, assays are always conducted in groups which include at least one known satisfactory product as a control. Eighteen percent of lots received during 1962 and the first six months of 1963 were examined. One-third of these lots failed to demonstrate minimum potency based on 66 percent pig protection.

**SUMMARY**

In summary, the relationship between the Biologics Control Laboratory, NADL, to AIQD is that of providing service. The functions of the laboratory are based on the quality control principle. Facilities have been operational for 18 months. During this period nearly all lots of products used in national eradication programs for three diseases, comprising 7-1/2 percent of the total number received, were examined. The rejection rate for this class of products was about 3-1/2 percent. Screening for potency was started and is continuing for products for seven other major diseases. These products account for about 35 percent of the estimated gross annual production of biologics. The average surveillance rate was 13 percent of lots received. The average rate of unsatisfactory findings was 19 percent of the lots tested.
REFERENCES

REPORT OF THE COMMITTEE ON BIOLOGICS

J. M. Hejl, Alexandria, Virginia, Chairman; N. H. Casselberry, Berkeley, California; R. P. Hanson, Madison, Wisconsin; R. V. Johnston, Indianapolis, Indiana; C. D. Van Houweling, Ames, Iowa; C. J. York, Bozeman, Montana

Your Committee on Biologics has considered several items relative to the production, testing and marketing of veterinary biologics. Our findings and recommendations are reported herein.

Item I - Progress in the development of pathogen-free animal tissues in which veterinary biologics can be propagated:

Your Committee agrees there is little question as to the importance of utilizing pathogen-free animal tissues in the propagation of biologics, especially those which contain living agents. The adoption of such requirement, however, appears impossible at this time.

Nevertheless, progress toward a goal of pathogen-free tissues is being made. Industry and research agencies are establishing specific pathogen-free colonies of chickens, pigs, mice, dogs, and other animals. For example, all eggs now being used for the production of poultry vaccines can be and should be free from certain salmonella and mycoplasma infections. Eggs free of RIF (Resistance Inducing Factor) can be obtained but the costs of such eggs are prohibitively expensive but we should not conclude that they will continue to be expensive in the future.

With regard to tissue culture products, a program is underway for the accumulation of classified cell lines. These lines being developed at several classification centers, will be made available to the American Type Culture Collection where they will be stored under liquid nitrogen and made available to commercial and research laboratories for a nominal fee.

Your Committee endorses and recommends the use of specific pathogen-free animal tissues for the production of vaccines to the extent practical; and these tissues should be free of those pathogens which are detectable at the present state of our knowledge. Government, Commercial and research laboratories are urged to intensity study of diagnostic methods which will broaden the scope of this activity.

Item II - Progress in stabilizing vaccines:

With improved freeze-drying equipment for processing vaccines and the accumulation in literature of information on the use of stabilizers, there has been much progress made in recent years in the stabilizing of vaccines. All dried vaccines can be and should be prepared with adequate stability for the label dating of such products. There is, however, a continuing need for basic research in factors influencing the degradation of micro-organisms.
Concurrent with such progress, we are seeing increased abuse of biologics by handlers and users. Storage of biologics without refrigeration, which appears to be the principle abuse, can negate all good expected of a previously potent product.

Your Committee recommends that licensed manufacturers of biologics, USLSA, AVMA, state and federal livestock regulatory officials, and others who are in a position to do so, should continue and expand education of those persons engaged in wholesaling, retailing, and using such biologics on the proper storage and handling of these products.

Item III - Biologics for use in eradication programs:

Biologics that are used in eradication programs must be subjected to additional evaluations such as is now being given to tuberculin, has always been given to Strain 19 vaccine, and testing is underway of hog cholera vaccines. When a biologic becomes an important part of the total eradication program, industry, institutions and Government should make certain that that product is as good as it can be and will do the job intended as well as any biologic will that is available. Strain 19 vaccine, for example, made an important contribution to the Brucellosis eradication program by reducing the susceptibility of animals that may become exposed to the infection. Prior to the use of Strain 19, this was found to be one of the great weaknesses in the eradication program. Entirely susceptible herds were set afire by the introduction of a small spark in the form of one infected animal. These fires rapidly spread through the highly susceptible herd whereas Strain 19 Vaccine, although not being 100 percent effective in establishing immunity, did establish a degree of resistance that slowed down the spread of the infection so that it was possible to get into these herds and "Clean them up" before the entire herd was infected and also contribute greatly in preventing the spread to neighboring herds in the community.

The old saying that a disease has never been eradicated with vaccination is probably true but it is also probably true that when a good immunizing agent is available and the eradication program is directed toward an established widespread disease, it is foolhardy not to take advantage of such an immunizing agent. This also further emphasizes the need for having the best biological products that can possibly be produced for protection against the disease that is being attacked. Of course, the incidence of disease in a given area must be taken into consideration before decisions can be made as to whether or not biologics should be utilized. A good example here is the difference in the way that hog cholera eradication is being attempted in the United States and has always been eradicated from Canada. Also, the experiences with attempted eradication of Newcastle disease in Canada and England without the use of the vaccines are indications of how it is wiser to make use of the vaccines when they are available than to try to do it without the use of biologics when the disease is widespread and already established.

A general statement cannot be made in regard to the use of vaccines in eradication programs; however, your Committee feels that each disease
situation must be evaluated individually. The potential of establishing an entity by the use of the biologics, the effect it will have on the diagnostic tests or diagnosis of the disease, the rapidity with which the disease spreads, and a number of other factors such as the area in which the disease occurs, the frequency of animal movements and traffic of people. All of these things must be taken into account and evaluated for each disease eradication program. In fact, in a country as large as ours, it is doubtful whether one policy can prevail throughout the country.

The hog cholera eradication program in the United States is another good example where eradication without the use of immunizing agents in some areas appears to be foolhardy and in other areas it is the thing to do, because of the extremely low incidence of the disease due, no doubt, largely to the very low swine populations in the same areas. Furthermore, the type of biological agent that is used may vary in different stages of the eradication program. Hog cholera again is a good example of where at one time it was feasible to use virulent virus and serum as a control measure. With the advent of the modified virus vaccines which are less dangerous from the standpoint of spreading disease they should be used in the early stages of an all out eradication program. However, it may well be that in the terminal stages no living hog cholera vaccines can be utilized and that the reliance will have to be placed in inactivated vaccines plus antiserum.

**Item IV - Need for and development of new biologics:**

The control or elimination of animal diseases never ends. As we control or eliminate old ones, new ones assume importance. Your Committee sees possible need for biologics for the following disease problems: Equine influenza, shipping fever complex, leukosis in chickens, bovine virus abortion, ram epididymitis, TGE syndrome in swine, virus diarrhea of cattle, and infectious keratoconjunctivitis.

Your Committee would like to call attention to another pressing problem, i.e., the need for certain biologics and diagnostic reagents, the demand for which is not great enough to attract competitive production, such as mallein, avian tuberculin, Johnin, Mycoplasma antigen, *Salmonella typhimurium* antigen and similar products. This is a problem that the state and federal governments and the biological products industry must work out a satisfactory solution.
THE DEGREE OF RESISTANCE AND THE INFLUENCE ON THE COMPLEMENT-FIXATION TITER PRODUCED BY INJECTIONS OF ANAPLASMOSIS ANTIGEN


Stillwater, Oklahoma

Although some control of anaplasmosis is now possible by identification of the carrier cattle by serologic test and by prevention of the infection with antibiotics, each of these methods has certain inherent difficulties that prevent their use in all situations. The development of a vaccine that would prevent anaplasmosis infection appears to be a more ideal solution for control of the disease. The preparation of such a vaccine has not been reported, but recent studies have indicated that a killed antigenic material will greatly reduce the severity of the disease although it does not prevent infection.

Kuttler \(^1\) has reported that an antigenic substance similar to the \(\text{CO}_2\) complement-fixing antigen, when injected into cattle 28 days prior to challenge, significantly reduces the anemia produced in the vaccinated animals. Kliewer \(\text{et al.}\) \(^2\) using a water lysed complement-fixing antigen, showed a similar reduction in the anemia of the vaccinated cattle following challenge. The antigens used by both workers produced serologic reactions in the cattle which were indistinguishable from those of anaplasma infection. If such a vaccine were to be commonly used in enzootic areas, the serologic reaction to the vaccine would be confused with the serologic reaction to the actual infection. The length of time that the complement-fixation test remains positive following vaccination becomes an important criterion for deciding between positive serologic reactions due to vaccination and due to infection. This report shows the variations in serologic titer following one and two injections of vaccine and later infection of the vaccinated animals with anaplasmosis.

MATERIALS AND METHODS

The antigenic material used for these studies was prepared by the Price method \(^3\) as modified by Miller. \(^4\) The freeze-dried antigen is suspended in light mineral oil to give 50 mg. of the antigenic material per one ml. of the oil.

Five two to 2-1/2 year old Hereford cattle were injected with 250 mg. of the antigen in August 1961, a second injection of 250 mg. of antigen was given on January 8, 1962 or 136 days after the first injection. The cattle were challenged with 10 ml. of anaplasma carrier blood on January 8, 1963 or 591 days after the first dose of antigen.

College of Veterinary Medicine, Oklahoma State University and the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.
Complement-fixation titers were performed on the serum from these cattle daily or weekly during the experimental period except for the last 3-1/2 months before they were challenged with anaplasma carrier blood. The packed cell volume and the percentage of anaplasma infected red blood cells were measured during the 55 days following challenge.

RESULTS

The changes in C-F titers observed in the cattle during the 1-1/2 years of the experiment are shown in Figure 1.

Complement-Fixation Titers Following Vaccination And Challenge

Figure 1. Changes observed in the C-F titers following vaccination and subsequent challenge with anaplasmosis carrier blood.

On the seventh day following the first dose of antigen, the complement-fixation test shows titers ranging from no reaction to 1:10. The titers increase rapidly to average 1:10, range from a trace to 1:160, on the ninth day. During the next ten days there is wide variation in the titers; however, one animal maintains a titer of 1:20 until the 127th day. From the 127th day to the 142nd day when the effect of the second dose of antigen appears, all of the cattle are negative or show only a trace reaction. After 142nd day the second dose of antigen causes rapid increase in the titer to a maximum average of 1:640, range 1:1280 to 1:160, on the 158th day. There is a rapid decline in titer to an average of 1:80, range from a trace to 1:320, on the 164th day or 28 days after the second dose of antigen. After the 164th day the average titer remains about 1:80 until the 217th day when there is a decline to an average titer of 1:20, range from a trace to 1:80. The titers remain at this level until the 392nd day of the experiment.
Due to the press of other work and technical difficulties no complement-fixation tests were run on these cattle from the 392nd day until the 501 day or one year after the second dose. On the 501 day of the experiment there are only trace reactions in the five cattle. The effect of the challenge of anaplasmosis carrier blood on the complement-fixation titers is first noted on the 512 day. The last complement-fixation titers noted on these animals show the average titer to be slightly over 1:640, range from 1:640 to 1:1280.

All of the cattle in this experiment became infected with anaplasmosis when challenged. Figure 2 shows the packed cell volume and percentage of anaplasma infected red blood cells as related to the time and magnitude of the antibody response following challenge. Anaplasmosis infected red blood cells appear on the 34th day after challenge, although there is a decline in the packed cell volume which starts about the 29th day. The maximum percentage of infected red blood cells occurs on the 41st day after challenge and reaches an average of about two percent with a range from less than one percent to 3-1/2 percent. The minimum packed cell volume occurs on the 45th day when there is an average of 21 percent with a range of 17 percent of 24 percent. During the period of anemia only one animal became ill for approximately two days.

Figure 2. The packed cell volume and percentage of anaplasma infected erythrocytes as related to the time and magnitude of the antibody response following challenge.
The results presented in this experiment and the earlier results of Kuttler and Kliewer indicate that the severity of anemia in anaplasmosis infection can be reduced by the types of vaccination described in these reports. Additional evaluations of such vaccines will be necessary before their use can be proposed as a practical method of reducing death losses from anaplasmosis.

Since the antibody reaction to the vaccine cannot be distinguished from the antibody reaction to the carrier state of anaplasmosis by the complement-fixation test, knowledge of the magnitude and the duration of the antibody response to the vaccine becomes important if the vaccine should come into wide usage. The animals in this experiment were followed over a period of 1-1/2 years and provide some information on the magnitude and duration of the antibody response to the vaccine.

These data indicate that the antibody response to the first dose of vaccine is relatively slight, irregular and of short duration. At no time during this first response do the five animals simultaneously show a 1:10 or greater titer. Only one animal in the group shows a 1:10 or greater titer after the 45th day following vaccination and it becomes negative to the complement-fixation test by 130th day. Four-fifths of these animals are not positive to the complement-fixation test after the 45th day. One of the animals shows a positive test until the 130th day.

The magnitude of the antibody response to the second dose of vaccine is comparable to the magnitude obtained in actual infection with anaplasmosis. The magnitude, however, decreases more rapidly than in infection with anaplasmosis. Where a positive reaction to the complement-fixation test may be expected to remain for years following infection, the positive reaction due to the vaccination has disappeared in all of the animals in this trial one year after the second dose of vaccine. One of the animals becomes negative to the complement-fixation test 2-1/2 months after the second vaccination. A second animal is negative by 5-1/2 months and a third by 8-1/2 months after vaccination. The other two animals become negative during the 3-1/2 months when no complement-fixation tests were performed.

The magnitude and duration of the antibody response to the vaccines reported by Kuttler and by Kliewer are in close agreement with the results reported here. It appears that cattle vaccinated by this method for anaplasmosis should not be tested by the complement-fixation test for the carrier state until a period of six months has elapsed from the time of vaccination. If the cattle are vaccinated a second time within six months, a year is required for the antibody levels to decrease below the point of positive reaction to the complement-fixation test. These are preliminary observations and more work is required before a definite time limit can be set for the positive reaction to the complement-fixation test as a result of vaccination. This study shows a marked variation in the antibody response to the vaccination as evidenced by the wide range of titers shown in Figures 1 and 2.
SUMMARY

Observation of the complement-fixation titers of five adult cattle vaccinated with 250 mg. of lyophilized anaplasma antigen in oil indicate a maximum titer of 1:160 and an average titer of about 1:10 following one dose of the vaccine. The duration of the positive titer to the complement-fixation test was about 45 days except on one animal which became negative about the 130th day. A second dose of vaccine five months after the first elicited a much greater antibody response, comparable to the magnitude produced by active anaplasmosis infection. This response required approximately one year to decline below the levels of detection by the complement-fixation test. Challenge of the 3-1/2 to four year old cattle one year after the second dose of vaccine produced anaplasmosis infection with less severe anemia and fewer clinical signs than would be expected in cattle of this age.

REFERENCES

REPORT OF THE COMMITTEE ON ANAPLASMOSIS

M. N. Riemenschneider, Oklahoma City, Oklahoma, Chairman; W. E. Brock, Stillwater, Oklahoma; G. T. Edds, Gainesville, Florida; T. E. Franklin, College Station, Texas; R. G. Garrett, Austin, Texas; D. Ibsen, Little Rock, Arkansas; K. L. Kuttler, Reno, Nevada; W. T. Oglesby, Baton Rouge, Louisiana, G. B. Rea, Salem, Oregon; T. O. Roby, Clarksville, Maryland; R. S. Sharmon, Washington, D. C.; W. L. Sippel, Kissimmee, Florida; E. H. Willers, Honolulu, Hawaii

Although research has developed methods for the control of Anaplasmosis, the disease continues to be a major problem of the cattle industry in extensive geographical areas of this country. One of the major developments for the control of anaplasmosis has been the production and use of serological tests to identify carrier cattle. In order to determine the extent and type of serological tests used, a questionnaire was circulated among the Chief Livestock Sanitary Officials. The questionnaire revealed that there are two serological tests in use, the compliment fixation (CF) and the capillary tube agglutination (CA). Present information indicates a high degree of correlation.

Sixteen states reported requiring the identification of serological positive cattle. At least two states (Hawaii and Minnesota) reported paying indemnity when cattle were required to be slaughtered. At least two states (Hawaii and New York) have imposed regulations pertaining to anaplasmosis.

The CF antigen is made available without cost from the Agricultural Research Service, United States Department of Agriculture (ARS-USDA) for research purposes, for diagnosis, control, screening procedures and field trials under a Memorandum of Understanding.

The Animal Disease Eradication Division (ADE) of the ARS-USDA reports that there are 22 laboratories engaged in routine testing of sera for anaplasmosis utilizing the CF test. These include Federal, State/Federal, and University laboratories located in 18 states. Some states have more than one laboratory conducting the test. The laboratories are located in Arizona, California, Hawaii, Idaho, Illinois, Kansas, Louisiana, Maryland, Mississippi, Montana, Nevada, Oklahoma, Pennsylvania, Texas, Utah, Virginia, Washington, and Wyoming.

Other States which have personnel trained in conducting the complement fixation test, but which are presently not conducting routine tests, are Arkansas, Connecticut, Delaware, Georgia, New Jersey, North Carolina, Oregon and Wisconsin.

The CA antigen is available commercially under state regulations as prescribed by special license issued by the USDA and has been evaluated on a limited basis at Maryland, Illinois and Nevada. The work was conducted on known CF sera to check the CA antigen for specificity. The results can be summarized in that the CA test was in agreement with the CF test at eighty to ninety percent.
In order to clarify some misunderstanding regarding the availability of the CA test, to veterinary practitioners and commercial laboratories, it is the opinion of this Committee, that no serological testing be conducted, except by an official laboratory. Unofficial testing has a tendency to stimulate the movement of carrier animals and thus spread disease rather than confine it.

The ARS reports that, "as far as field trials on anaplasmosis control are concerned, we are still cooperating with the same groups and carrying on the same studies as outlined last year. There is one exception to this, in that the work in Wyoming has been discontinued. The other field studies are proceeding at Kerrville, Texas, Jeanerette, Louisiana, Stoneville, Mississippi and Reno, Nevada. Detailed research reports on these activities will be made from time to time. Current progress is similar to that of last year." Also, they report that some states have adopted and offered voluntary anaplasmosis control programs to cattle owners within their borders but the extent of these activities are not presently known to the Division.

The state veterinarian, Dr. E. H. Willers of Hawaii, states that in his opinion Anaplasmosis has been eradicated from the cattle herds of Hawaii by the use of the CF test. The chief problem from now on will be to prevent re-introduction through imported carrier animals. His report is as follows:

"During fiscal year 1962-63, 6,487 complement-fixation tests for anaplasmosis were conducted on dairy cattle in Hawaii. Only one animal, recently imported, was classed as a reactor. This animal had been negative on the import quarantine test but gave a 4+ reaction on the 60 to 90 day retest. The serological diagnosis was confirmed by calf inoculation trial.

"Out of 4,642 tests that were conducted on beef cattle, three gave 4+ reactions to the Complement-fixation test. One of these was a heifer consigned to a feedlot that was slaughtered without benefit of retest; the other two were on eight month old Angus bull calf that was found negative on retest, and a five year old cow that was still on the retest list as the year ended. Seven cattle out of 1,561 imported during the year were classed as reactors on entry test. In each instance, the test results were confirmed by calf inoculation trials. Three other imports showing suspicious reactions were retained in quarantine pending retest results as the year ended."

The Committee wishes to state that there is evidence of continued interest in the disease by Livestock Sanitary Officials, cattle owners and research workers. Some States are making available control programs on a voluntary basis. Additional studies are being conducted attempting to develop a vaccine.

The Committee wishes to make the following recommendations:

1. That adequate funds be provided to carry out the following research:
   A. The study of known vectors and their control as well as a search for other possible vectors. That such research be expanded by
close cooperation between the various animal disease workers and entomologists.

B. That intensified research be continued and expanded to determine the exact nature of the causative agent. It is obvious that without this information the production of an immunizing agent, a complete understanding of the pathology of the disease, successful treatment and control are difficult, if not impossible.

C. The Committee believes there is an urgent need for continued and broadened experimental studies in the various geographical areas, and recommends that such steps be taken. By expanding the efforts, we may extend our knowledge of the vectors and reservoirs of infection. This information would contribute greatly toward developing feasible control procedures.

D. That research be continued to improve and further evaluate the CF and CA tests and encourage the development of other simplified economical tests.

E. That efforts be made to develop an effective inexpensive treatment for acute and carrier animals.

F. That continued and expanded research be conducted to develop a practical immunizing agent.

2. That any anaplasmosis immunizing agent developed which produces a serological reaction only be used officially under the authority of the Chief Livestock Sanitary Official.

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

4. That the ARS continue to make available sufficient anaplasmosis antigen to supply the cooperating laboratories and research workers.

5. That the ARS continue and expand their training program for serologists in order that adequate trained personnel be available to all possible cooperating laboratories.


7. The Committee recommends that the United States Livestock Sanitary Association publish a brochure entitled "What is Known About Anaplasmosis" and that this committee be authorized to prepare this publication. This report is to be prepared for the livestock industry. Such a booklet could fill a real need for cattle owners.

The Anaplasmosis Committee urges that every possible effort be made to implement the recommendations in order that we may reduce the economic loss to the cattle industry brought about by this very devastating and complex disease.
RESOLUTION RE: ANAPLASMOSIS

WHEREAS, it has been brought to the attention of the Committee on Anaplasmosis of the United States Livestock Sanitary Association that there is a noticable lack of practical, factual type of information available to the livestock owners; and

WHEREAS, the Anaplasmosis Committee wishes to make available, practical information which can be used by the livestock industry to aid in the control of anaplasmosis; and

WHEREAS, the Anaplasmosis Committee does have available to it, a great deal of research information, much of which can be put to practical use;

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Association authorize and sponsor the publication of a brochure entitled "What is Known About Anaplasmosis;" and

BE IT FURTHER RESOLVED, that the Anaplasmosis Committee of the United States Livestock Sanitary Association be responsible for preparing this publication.
THE CONTINUING PUBLIC HEALTH IMPORTANCE OF BRUCELLOSIS

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Brucellosis is the prime example of a disease of domestic animals which is readily transmissible to man. It does not spread from human to human with the possible exception that the nursing child may occasionally become infected through the mother’s milk. Brucella organisms do not multiply in nature outside of an infected animal. Each case of human brucellosis is due to an individual instance of the transfer of Brucella organisms from an infected animal to man. While an occasional case of human infection has been related to infection in wild animals, for instance the caribou in Alaska, there is no evidence that any significant number of human cases arise from these sources. The infected animal source is confined for all practical purposes to the domestic animals, cattle, swine, and goats. In the United States the goat population is not large. Infection in goats has been a minimal problem confined to a few southwestern states. Our major concern then is with cattle and swine.

The routes of transfer of infection from animal to man are well known. Brucella organisms may be shed in the secretions and excretions of infected animals. They may be present in the blood, lymph glands, the reproductive tract, and many other tissues. Infection of man occurs through the skin, mucous membranes, wounds, abrasions, and the digestive and respiratory tracts. Direct contact with infected animals, their tissues, or their environment, or consumption of unpasteurized milk or other dairy products provide the opportunity for the organism to enter man.

Brucellosis in man is a preventable disease. As long as we have Brucella infection in our domestic animals, people will continue to contract the disease. When brucellosis has been completely eradicated from our domestic animals, the disease in man also will disappear.

In years past some fifty percent of all cases of human brucellosis in the United States were due to consumption of unpasteurized milk. A large share of the general population was regularly exposed to the disease. In the last ten or fifteen years the situation has changed significantly. Most people now use pasteurized milk. The consumption of raw milk is confined largely to rural areas and to the farms of origin. Infection in an animal, or a herd, results in exposure by this route of but a single family. Ninety percent or better of all human brucellosis is due to direct contact with infected animals or their tissues. Brucellosis has truly become an occupational disease. The people exposed are primarily the cattle and swine breeders and raisers, their families and employees, the veterinarian, the butcher, and others who handle infected animals or their

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The public health problem we are talking about then is confined mainly to those people who derive their livelihood from the animal industry. In our promotional efforts in the eradication program this fact should be made abundantly clear at every opportunity.

Reported cases of human brucellosis hit a peak in 1947 of 6,321 cases. The reported incidence has declined markedly since then. In 1956 there were 1300 cases; in 1961, 636. Preliminary data for 1962 lists 429 cases.

While the disease in man due to the cattle type of the organism, *Brucella abortus*, is continuing to decrease, that due to *Br. suis*, the species contracted from swine, continues at about the same level. This is now the principal source of the occupational disease in packing plant employees. There will be no decline in disease due to this species until the disease in swine is brought under control.

Brucellosis has always offered diagnostic difficulties, and many of us believe that the true incidence has been considerably in excess of that reported. In the distant past, say 20 years ago, diagnostic criteria were less rigid than at present and many patients with chronic illness were given a diagnosis of brucellosis on inadequate grounds. With the establishment of diagnostic criteria for the disease, improvements in diagnostic laboratory techniques, and a better informed medical profession, the reported cases have been better substantiated. Currently, with the activities of the Communicable Disease Center in investigating the basis for diagnosis of reported cases, we may feel reasonably sure that these figures represent true cases of the disease. However, in practice, many patients with various acute infections are currently being treated with antibiotics without a definitive diagnosis being made. This undoubtedly obscures the diagnosis of brucellosis in many cases. Further, with the lessening in incidence of the disease, the possibility of brucellosis is considered less often in the differential diagnosis of febrile illness. Cases are now being missed because of the lack of familiarity of the younger generation of physicians with this disease. In both of the above instances, unless the patient continues to be ill, or develops a complication or chronic form of the disease and a diagnosis is ultimately made, the cases do not appear in the reported figures. Also, reporting depends upon the individual physician and is seldom complete for any disease. The true incidence of human infection is not known. It is certainly in excess of the reported figure and may be several times higher. In defining the size of the public health problem offered by the disease, these facts must be kept in mind.

In order to assess the public health importance of brucellosis it is necessary to consider not only the incidence, but also the nature of the disease. Brucellosis is not the common cold. It is a disease which may be chronic and debilitating, and serious complications may occur.

Acute brucellosis is an incapacitating disease lasting for several weeks or months. The chronic and localizing forms are of even greater concern. Recurrent or relapsing acute illness, or continuous low grade debilitating disease may persist for years. In many instances these patients are unable to perform useful work to earn a livelihood for long periods. Such active infection has been known to last for as long as 10, 15, or
even 25 years. Many patients develop psychiatric syndromes, as a direct result of their infection, or of their reaction to chronic illness. Some become alcoholics, narcotic addicts, or suicides. Prior to the era of antibiotic treatment, in some 20 percent of the cases the acute illness was followed by relapsing or chronic disease. You may recall such instances in your own neighborhoods. The suffering and economic hardships experienced by these people were appalling. The disease has not changed its nature. Chronic brucellosis still occurs. Undiagnosed, misdiagnosed, and inadequately treated cases still result in chronic illness.

Of particular significance are the cases with local destructive lesions. In some series of cases, from 12 to 14 percent have had such lesions. Localization may occur in any organ or tissue, most commonly in the bones, producing osteomyelitis. The lumbo-sacral spine and pelvic bones are sites of predilection. Destruction of an intervertebral disc and adjacent vertebral bodies, with partial collapse and gibbous formation, produces instability of the spinal column and pressure on nerve roots. There is severe pain and threat of paralysis. Surgical fusion of the spine is necessary. Even so, these patients are left with residual disability for the rest of their lives.

Such local destructive lesions may involve other major structures, the long bones are frequently affected; chronic suppurative kidney infection, pyelonephritis, occurs; infection of the heart valves, always fetal before the advent of antibiotics, is a serious complication, as is liver damage leading to death from cirrhosis of the liver. Lesions in the lung have been confused with carcinoma of the lung. Suppurative arthritis of a large joint, as the hip or knee, produces irreparable damage and resulting disability. We could list further instances as any part of the body may be involved, but these examples should suffice to elucidate this aspect of brucellosis.

The greater proportion of these local destructive lesions are due to Br. suis, the organism usually contracted from swine. For this reason Br. suis infections assume an importance out of proportion to the incidence of the disease. Hence, the need to control and ultimately eradicate swine brucellosis is readily apparent.

What effect so far has the brucellosis eradication program had on the incidence of human brucellosis? The proportion of the total drop in incidence directly due to the program activities is difficult to assess. In 1957 I subjected the annual incidence figures for the past decade to statistical analysis, attempting to correlate decline in incidence in the various states with progress in the eradication program. Several major factors were operative during this period to reduce the reported incidence. The bovine brucellosis eradication program was in an accelerating phase, the percentage of people drinking pasteurized milk was increasing, and the broad spectrum antibiotics had come into widespread and indiscriminate use. This latter factor tends to obscure the diagnosis of brucellosis and thus lower the reported incidence. While the data were suggestive of an effect in some states, there was no clear cut proof that the decline in bovine brucellosis resulting from the eradication program had yet been
reflected in the human incidence figures. In this complex situation, the lack of statistical proof does not mean that no portion of the drop in human incidence prior to 1957 was due to the eradication program. Perhaps a significant proportion should be so credited.

The further drop in incidence since 1957 is easier to evaluate. The degree of pasteurization of milk and the use of antibiotics have been relatively constant factors. Aside from natural fluctuations of disease incidence, the further decline in human cases may be justly attributed to the results of the eradication program. On this basis, we arrive at a minimum figure for the past year of approximately 800 cases of disease which did not occur, presumably as a result of the progress made in the eradication program since 1956.

This figure of 800 cases is based on the decline in reported incidence. In accord with previous reasoning on the true incidence of the disease, perhaps it should be considerably larger. If a sizable proportion of the drop prior to 1956 can be justly included, the total annual saving in cases resulting from the eradication program would be much larger. Now consider the percentage of these cases which might have been of the more severe, chronic, or disabling type. A minimum estimate would be 10 percent. The figure (whatever its size) represents but a single year. Unless the eradication program founders, this is a permanent gain. Project it over a period of years and the saving in both total cases and disabling cases becomes truly impressive.

It is impossible to place a monetary value on human suffering and disability, but in my opinion, economic factors aside, the reduction in incidence of human disease, already brought about by the brucellosis eradication program, amply justifies all the money, time, and effort so far expended.

What is the size of the problem that remains? From the reported incidence, some 400 to 500 cases annually. As I have already intimated, the true incidence may be several times higher. If we now consider the serious nature of the disease, and the fact that these cases are confined to a relatively small segment of the population, the figures assume greater significance. For the groups at risk, the people working in the animal industry, the threat of human infection still remains, and constitutes a potent argument for intensification of the brucellosis eradication program and ultimate elimination of this disease, from our domestic animals, and from our people.

REFERENCES
STATUS OF STATE-FEDERAL BRUCELLOSIS
ERADICATION PROGRAM
Hyattsville, Maryland

In our reports to this Association each year we have reviewed accomplishments since the previous meeting and weaknesses in the program that should be strengthened. Last year attention was called to the need for intensifying the eradication efforts in some areas of the country if nationwide certification was to be achieved by the end of fiscal year 1965. This problem is even more acute now than it was at the time of our last report, and unless the lagging States are able to advance their certification program more rapidly the 1965 goal will not be realized. If the present trend continues, there is a strong possibility that at least six States will not complete their certification programs by that time. Figure 1 shows those areas in the nation where progress has been delayed.

*Senior Staff Officer, Cattle Diseases, Agricultural Research Service, United States Department of Agriculture.
The 1965 date is still considered a realistic goal for certifying the entire country and with increased effort in a few areas it can be reached. Everyone concerned should understand that unless this program is completed soon it may become increasingly difficult to continue the program at effective levels. At this decisive stage of the program, therefore, it is imperative that maximum progress toward eradication be made in all areas of the country.

The gap between the most active and the least active States continues to widen in spite of efforts to alert the livestock industry in the lagging states to the urgency for identifying and eliminating all remaining pockets of infection as rapidly as possible. By moving ahead in all areas, progress is more easily maintained and eventual eradication of the disease from the nation is advanced. In this regard, it is essential that dangers associated with the movements of cattle from noncertified areas be realistically assessed. We cannot afford to jeopardize the progress already made in most areas of the nation by movements of potentially infected or exposed animals.

Another problem that is becoming increasingly serious from the standpoint of achieving total eradication is the official vaccinate that has contracted brucellosis in spite of vaccination. The masking effect of vaccine together with the titer tolerance now given official vaccinates is contributing in some areas to perpetuation of the disease. This threat to the successful conclusion of the cooperative brucellosis eradication program needs to receive serious consideration. To illustrate this need, approximately one-half of all cattle from which field strains of *Brucella abortus* have been isolated under the expanding "problem herd" program have been official vaccinates. Serious outbreaks of brucellosis have occurred in Certified Brucellosis-Free Herds when young vaccinated animals have been purchased from previously infected herds that were later declared brucellosis-free on the basis of tests conducted on adult animals only. This has happened in spite of the fact that some of the receiving herds were 100 percent vaccinated. Investigations frequently disclose that vaccinated heifers had been pastured with animals, later proven to be infected, which became parturient in the presence of the vaccinated heifers. A number of outbreaks have resulted also from purchases of vaccinated heifers from herds of unknown status.

Critical surveys have been made to determine the brucellosis status of cattle moving from certified and noncertified areas in many parts of the country. These data showed a marked difference between the levels of infection disclosed, depending upon the respective areas of origin involved. For example, only one reactor was found for each 2,475 cattle tested from certified areas. This contrasts with the one reactor disclosed for each 152 cattle tested from noncertified areas. These results emphasize two points that should be considered in furthering the eradication effort. One of these relates to the safety of allowing considerable freedom of movements from the certified areas, while the other point concerns the importance of exercising maximum care in qualifying animals of unknown status that originate in noncertified areas.
The practicality and effectiveness of screening procedures in the brucellosis eradication program have been clearly demonstrated. There is no question about the important roles ring testing and market cattle testing are playing in attaining final eradication of brucellosis. While satisfactory levels for ring testing are being established in most sections of the country, there is still a need for considerable expansion of market cattle testing activities. Great strides have been made in the degree of cooperation existing between States under the ring testing and market cattle testing programs.

CERTIFICATION OF AREAS

It is a pleasure to report that Kentucky achieved Statewide Modified Certified Brucellosis Area status as of August 30, 1963. This brings to 33 the number of States which, together with Puerto Rico and the Virgin Islands, have been so designated. Of all States east of the Mississippi River, only five—Ohio, Illinois, Florida, Alabama and Mississippi—have yet to initially certify. States west of the Mississippi River which have not yet attained Statewide certification are North Dakota, South Dakota, Montana, Wyoming, Nebraska, Iowa, Colorado, Arkansas, Texas, Louisiana, Alaska and Hawaii. Figure 2 depicts the current situation with regard to area certifications.
In spite of the fact that only 555 counties have yet to achieve Modified Certified Brucellosis Area status in the United States, the number of counties newly certified each year remains fairly high. For instance, a net of 167 new counties achieved this status during fiscal year 1963. There were 17 counties which lost certified status during the year, but 15 of them have been reinstated. There were three States—Missouri, Kansas and California—which attained Modified Certified Brucellosis Area status during fiscal year 1963.

In the Certified Brucellosis-Free Area program, the number of counties added each year is increasing at a satisfactory rate. During fiscal year 1963, 74 free counties were added as compared to 56 in 1962. At present there are 221 Certified Brucellosis-Free Counties in 18 States, Puerto Rico and the Virgin Islands. Arizona and Nevada also have Certified Brucellosis-Free Indian Reservations, and both States expect to add entire counties soon.

Combining the modified certified and free counties, 82.3 percent of all counties in the United States have attained certified status. An additional 132 counties are engaged in an area program leading to certification in the near future. Only 423 counties have yet to initiate organized certification efforts. Area certification accomplishments are shown graphically on a percentage basis in Figure 3.

The procedures for establishing and maintaining Certified Brucellosis-Free Areas are satisfactory, as proved by the fact that these areas, once

**BRUCELLOSIS ERADICATION**

**COUNTY CERTIFICATION STATUS**

- Area Work in Progress: 125 (4.0%)
- Brucellosis Free: 217 (6.9%)
- Modified Certified: 2,378 (75.4%)
- Individual Herd Participation: 433 (13.7%)

Total 3,153

AUGUST 31, 1963

U. S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
established, are experiencing no difficulty in maintaining their free standing. Based on past records, it is predicted that no more than one brucellosis outbreak per free county will occur each two years. If this record can be maintained until all States have achieved initial certification, it is certain that the eradication of brucellosis will be a reality shortly thereafter.

EFFICACY OF STRAIN 19 VACCINE

A survey of brucellosis outbreaks in modified certified range and semi-range counties has been underway since March of 1961. A preliminary summary was included in my report to this organization a year ago. The purpose of the survey is to assess the efficacy of Strain 19 vaccine in limiting the spread of brucellosis in certified range and semi-range counties. Detailed information on all infected herds is submitted at the time the counties request recertification. Fifteen States have submitted data for the survey to date—Arizona, Arkansas, Colorado, Florida, Idaho, Mississippi, Montana, Nevada, New Mexico, North Dakota, Oregon, South Dakota, Utah, Washington, and Wyoming. In all, 145 counties are included.

The cattle population of the participating counties is 4,090,961 animals in 108,976 herds. About 35 percent of the herds have been represented in blood or ring tests during the three years of area certification. Brucellosis has been found in 1,434 herds (3.8 percent of those tested).

In the infected herds there were 83,293 cattle (the exposed population). Thus, 9.1 percent of the cattle tested were exposed to brucellosis during the three-year period. Of the exposed cattle, 26,213 had been vaccinated. (31.5 percent). Of these, 558 were reactors—a brucellosis incidence of 2.13 percent. This approximate percentage of reactors among exposed vaccinated populations has been apparent in a number of field surveys.

There were 69,074 beef cattle in infected herds, and only 19,384 of these had been vaccinated (28.1 percent). There were 263 vaccinated beef animals which were reactors (1.4 percent of the vaccinated beef population). On the other hand, 7.6 percent of the nonvaccinated beef animals were reactors. By a simple calculation it is found that there were approximately 81.6 percent fewer reactors among the vaccinated population than among the nonvaccinated. This can be interpreted as the apparent protection afforded by the vaccine in infected beef herds in certified areas.

In those infected beef herds in which only part of the animals were vaccinated, 1.3 percent of the vaccinated animals were reactors, compared with 7.2 percent of the nonvaccinated animals. In these herds, the apparent protection afforded by the vaccine was 82.0 percent.

Brucellosis was found in 18 beef herds in which all of the animals were vaccinated. These herds contained 1,052 animals of which 2.3 percent were reactors. Although this survey will be continued, the data collected each year tend to confirm the view that vaccine alone will control but not eradicate bovine brucellosis in either range or dairy areas.

In the cooperative State-Federal brucellosis eradication program, vaccine will continue to play an important role for those sections of the
country where the incidence of infection is still relatively high. However, its value in other areas will decline as eradication is approached and screening devices become fully operative. Withdrawal of vaccine under these conditions will permit the disease to be identified more readily and will facilitate elimination of the last infected animals.

NEWLY INFECTED HERDS IN CERTIFIED STATES

For three years certified States have been submitting details on all outbreaks of brucellosis in herds which have not revealed reactors for more than a year. Such herds are referred to as "newly infected." There are 26 States plus the Virgin Islands and Puerto Rico which have been submitting data for a full three years, and comparisons in this group provide an index as to the efficacy of present program procedures, as well as a probable time-table for total eradication.

Newly infected herds were found in 64 percent of the counties in these States during 1963. This compares with 67 percent in 1962, and 70 percent in 1961, and demonstrates a significant downward trend.

There were 3,976 newly infected herds in 1963, down 11 percent from the 4,479 disclosed in 1962. The 1962 total was down only one percent from 1961. Therefore, the progress reflected in 1963 is highly significant. This progress is being made in the face of increased program efficiency through intensified screening procedures including the market cattle and milk ring testing programs. These procedures have a tendency to raise the number of infected herds being found each year.

The average number of animals in newly infected herds has remained at approximately 30 head during the entire three-year period. The number of reactors found in these herds has remained almost constant at approximately 9,000 each year. The percent of reactors in the newly infected herds has varied between 6.5 and 7.5, with the highest percentage in 1963.

The percentage of newly infected herds with a history of prior infection is decreasing materially from year to year. In 1961, 33 percent had revealed prior reactors; in 1962, 28 percent; and in 1963, only 23 percent.

The number of newly infected herds revealing only one reactor on the initial herd test decreased significantly in 1963. In 1962, there were 3,132 singleton reactor herds, while in 1963 there were only 2,724. This is a decrease of 13 percent, and should alleviate some of the fears which have been expressed concerning the singleton reactor herds. These herds averaged 26 head, while multiple reactor herds averaged 39. Additional information is being gathered to assess this situation, and further reports will be forthcoming in this regard.

BLOOD TESTING

During fiscal year 1963, 1,915,816 lots of cattle were blood tested. This is an increase of 24 percent over 1962, largely due to expanding activity under the market cattle testing program.

The number of infected lots increased from 59,571 in 1962, to 68,419 in 1963. Most of this increase can be attributed to the effective market
cattle testing program initiated in a single State during July 1962. This State identified reactors in 18,604 lots, most of which were tested at livestock markets.

Figure 4 shows the trend in blood testing activities and infection levels for fiscal years 1935, 1945, 1955 and 1963. The number of cattle blood tested in the United States during fiscal year 1963 decreased again—this year by almost 700,000 tests. However, the number of reactors increased 5,531 for a total of 132,370. This is 1.17 percent of the animals blood tested, and compares with 1.06 in 1962. This increase was predicted some time ago, and represents increased program efficiency through the screening programs, decreased "on-the-farm" testing, new activity in areas with a relatively high incidence of brucellosis and other minor factors. Because of the changing character of the program, yardsticks used to measure program accomplishments in the past no longer apply, and new criteria must be considered.

OFFICIAL CALF VACCINATIONS

The number of calves vaccinated during fiscal year 1963 was 7,076,668. This is a new record for the United States, and represents an increase of 336,324 over 1962. It is estimated that approximately 55 percent of the eligible calves were vaccinated throughout the nation in fiscal year 1963.
In spite of the fact that many States are increasing the frequency of milk ring test rounds, the total number of such tests continued to decrease. The decrease for 1963 was more than five percent. A total of 1,632,591 tests was conducted. Of these, 19,497 were suspicious—1.2 percent. This is a decrease of 25 percent from the 1.6 percent suspicious rate of 1962. The marked reductions in the percentage of ring test suspicious herds is shown in Figure 5.

The majority of milk ring test suspicious herds reveal blood test reactors, and this test continues to be one of our most efficient means of finding infected herds. However, the market cattle testing program may soon take the lead in this respect.

Under the "problem herd" program, the milk ring test is proving more and more specific. Animals responsible for persistently suspicious milk ring tests of herds, even in the absence of blood reactors, are frequently found to be Brucella shedders, and their elimination usually solves the suspicious ring test problem.

![Figure 5: Brucellosis Eradication: Ring Testing Milk, Herds](image-url)
Brucellosis Eradication

MARKET CATTLE TESTING PROGRAM

COWS BLOOD TESTED

<table>
<thead>
<tr>
<th>FISCAL YEAR</th>
<th>1961</th>
<th>1962</th>
<th>1963</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>803,912</td>
<td>1,833,962</td>
<td>2,493,754</td>
</tr>
</tbody>
</table>

- Packing Plants (Slaughter)
- Concentration Points (Slaughter and Feeder)
- Concentration Points (Breeding and Replacement Cows)

MARKET CATTLE TESTING

Figure 6 depicts recent advances made in the market cattle testing program. Activities in this program increased significantly during 1963 when 2,493,754 tests were recorded. This is an increase of 36 percent over the 1,833,962 tested in 1962. Although the market cattle testing program is expanding considerably, it is still lagging behind the desired adoption schedule. There is a potential of at least 8,000,000 tests per year when the program becomes fully operational, so we have only reached about 31 percent of the goal.

Many market animals with significant titers were traced to herds of origin and many of these herds were tested. In all, 4,314 herds of origin were reported tested, and 1,506 (35 percent) revealed additional reactors. There are indications that it is advisable to conduct immediate tests on herds of origin and a subsequent 30 to 45 day retest in order to avoid overlooking animals in early stages of the disease.

It is interesting to compare the results of tests of herds of origin of slaughter and feeder cattle with results of tests of herds of origin of breeding and replacement animals. Out of a total of 2,416 herds of origin of slaughter and feeder animals, 731 revealed additional reactors. When
reactors were found, the incidence of the disease within these herds was 8.4 percent. On the other hand, 1,898 herds of origin of breeding and replacement reactors were tested and additional reactors were revealed in 775. In the infected herds, there were 3,977 reactors—an incidence of 12.7 percent. This is significantly higher than the incidence in the infected herds of origin of slaughter and feeder cattle.

A number of States have adopted the market cattle testing program as a means of initially certifying and recertifying counties as Modified Certified Brucellosis Areas. These are shown in Figure 7. To date, the number of counties which have submitted requests for initial certification under the market cattle testing program is limited. However, it is believed that this procedure has the general approval of the livestock industry in all areas where the program has been adopted, and will expand.

"PROBLEM HERD" PROGRAM

Additional brucellosis epidemiologists were trained and assigned during fiscal year 1963. These men continue to make a vital contribution
during the final phases of brucellosis eradication. There is a very small number of infected animals which do not respond at diagnostic levels to the standard blood tests. Procedures followed under the "problem herd" program have been effective in identifying such animals in all instances and freeing the herds of brucellosis.

There have been a few problems; for instance, a limited number of farmers have withdrawn from the program rather than give up particularly valuable animals. A few others have dropped out because they did not fully understand the benefits of the program, nor its operation, prior to initiation of supplemental testing activities. However, the number of such withdrawals in the United States to date have been extremely small. This speaks well for the excellence of the informational effort which precedes initiation of "problem herd" procedures.

**HUMAN BRUCELLOSIS**

The number of cases of undulant fever reported to the U. S. Public Health Service continues to decrease significantly each year. For instance, in 1961 there were 636 cases, but during 1962 (calendar year) there were only 409. This is a decrease of 94 percent from the 6,321 cases reported in 1947. The striking correlation between the reduced incidence of human and bovine brucellosis is reflected in Figure 8. If there were no other

**BRUCELLOSIS ERADICATION**

![BRUCELLOSIS-UNDULANT FEVER](image-url)
benefits from the cooperative State-Federal brucellosis eradication pro-
gram, this one would be sufficient to justify our efforts.

There was a time in the not too distant past when all veterinarians
entering private practice faced the probability of contracting brucellosis
if they treated large animals, especially cattle. Today only a relatively
few large animal practitioners will contract brucellosis during their life-
time. Certainly this is one outstanding instance where a profession has
improved its lot considerably through its own disease eradication efforts.
Although it has not been definitely determined, it is believed that almost
as many cases of brucellosis in veterinarians are now caused by inocula-
tion accidents involving Strain 19, as result from exposure to virulent
Brucellae in the field.

SWINE BRUCELLOSIS ERADICATION

Interest throughout the swine industry continues to increase in rela-
tion to establishing Validated Brucellosis-Free Herds of swine. Your at-
tention is called also to a new publication of the Agricultural Research
Service entitled, "Validated Brucellosis-Free Herds." It is a listing of
validated herds throughout the United States. At the time of publication

States With Validated Brucellosis-Free Swine Herds
September 1, 1963

Figure 9
there were 937 Validated Brucellosis-Free Herds throughout the United States. These are listed in the booklet by herd owner, town, rural route, county and breed of swine. It is anticipated that this listing will be re-issued at frequent intervals as changes and additions are accumulated. There have been many new validated herds added since the booklet was prepared, for a current total of 1,060. Figure 9 identifies those States in which validated herds are known to exist.

Such a listing is particularly useful in view of the fact that 27 States now recognize by regulation or law Validated Brucellosis-Free Herds as sources of breeding swine qualified for importation without further testing. Two years ago, only 15 States recognized such swine on the same basis. I recall no instance in which a recommended procedure is being more quickly adopted than in the case of accepting swine from validated herds. Such desirable incentives offered to swine breeders will hasten the day when swine brucellosis will be eradicated.

Figure 10 compares the results of blood testing swine for brucellosis in fiscal years 1956 and 1963. It is interesting to note the increased number of herds tested and the significant reduction in infection levels for the two years involved. In 1956, 9.5 percent of the 14,919 lots tested contained reactors; in 1963, only 2.5 percent of the 49,947 lots tested were infected. It is anticipated, however, that the percentage of reactors may rise for a time as the swine brucellosis eradication program is expanded. Much
progress has been evident in the tested segment of the swine population over the past several years, demonstrating the soundness of the recommended porcine brucellosis eradication procedures. However, it is believed that these data are not representative of all swine breeding herds, but restricted primarily to purebred herds and show stock. The commercial breeding herds must be reached in order to move ahead in the program. Market swine testing programs, accompanied by improved animal identification systems, would pay great dividends pending the ultimate eradication of the disease from all herds.

Field trials are being conducted in a number of States to evaluate a rapid new screening test for swine brucellosis. The test requires only a few drops of blood from each animal, can be applied on the premises, and appears to be an efficient and practical means of identifying Brucella suis infected swine herds. If this test continues to prove comparable to the standard tube and plate tests at screening dilutions, there is no limit to its application when incorporated into complete area swine brucellosis eradication programs.

BISON BRUCELLOSIS

Brucellosis in bison has been found to be a serious problem for consideration in completing the final phases of the bovine brucellosis eradication program. It has been conclusively demonstrated that some of the bison herds are extensively affected with the same type of Brucella abortus that normally infects cattle. Therefore, the hazards associated with direct or indirect contacts between healthy cattle and Brucella-infected bison are obvious. While the number of infected bison may not be great, their continued presence in areas where active efforts are being made to eradicate brucellosis constitutes a situation that should not be tolerated indefinitely. An attempt to minimize this hazard is embodied in a proposed Federal regulation dealing with movement of bison interstate.

How soon brucellosis in bison will be eradicated is uncertain at this time. However, it must be accomplished eventually. All States are urged to establish adequate and reasonable safeguards to limit the transmission of brucellosis from bison to cattle as soon as possible.

GENERAL OBSERVATIONS

With 82 percent of all counties now certified, it is essential that everything possible be done to complete the nationwide certification effort by the end of fiscal year 1965. Until this is accomplished, maximum progress toward final eradication will be delayed. At this stage of the program we should not allow 555 counties to interfere with further progress in the 2,598 certified counties.

There is an urgent need to reduce the permitted age for official vaccination to the lowest level consistent with the establishment of servicable protection. There is evidence that vaccination at three months of age will provide protection comparable to that afforded by vaccination at higher
ages. Efforts need to be accelerated to substantiate this indication through additional controlled studies. Vaccinal titers associated with higher age vaccinations are causing increasingly difficult problems as the incidence of infection is reduced to low levels.

The "problem herd" program has developed to the point where it is highly effective and should become an integral part of the eradication program in all States that have attained full certified status. The assignment of qualified epidemiologists to interested States will continue as rapidly as they can be developed through available training facilities.

The rapidity with which final eradication of brucellosis is accomplished will, in the long run, depend largely upon the effectiveness of surveillance procedures. In combination, the brucellosis ring testing and the market cattle testing programs provide the tools for complete coverage of both milking and non-milking cattle herds. The value of these procedures have been substantiated fully on the basis of field application. It only remains for them to be utilized in such a manner as to provide complete and effective coverage. When this has been accomplished, along with appropriate handling of presumptively infected herds, early completion of the eradication program will be assured. Positive identification of all mature female cattle moving in channels of commerce would materially help also in controlling the spread of brucellosis as well as other contagious and infectious diseases.

Too frequently we learn of reactors being retained for calving before they are slaughtered. Such animals are likely to shed billions of virulent Brucella organisms at the time of calving and expose an undetermined number of contact animals. At this stage of the brucellosis eradication program every effort should be made to move reactors to slaughter as promptly as possible regardless of pregnancy status. Cattle which are not showing signs of impending parturition are usually passed for food without restriction under Federal Meat Inspection. Even though evidence of early calving may result in the carcass being passed for cooking only, in most instances the maximum indemnity would be paid anyway. Any minor losses that might be involved in cases of this type would be more than compensated for by eliminating the danger of further spread of the infection at calving time. Although maternity stalls help minimize this hazard, they are never fool-proof and are difficult to operate effectively.

Consistent with the goal of final eradication, maximum freedom of movement should be granted cattle originating in certified areas. By the same token, movements of cattle from herds of unknown status in non-certified areas should be restricted to the extent necessary to provide maximum protection for cattle herds in the certified areas. We cannot afford to jeopardize the progress made in most parts of the country by movements from areas that have made little or no effort to combat brucellosis.

When the alternate titer interpretation for official vaccinates was adopted in 1955, there was full justification for this action. At that time there was an urgent need to expend the level of vaccination, especially in areas of relatively heavy infection. This action served a useful purpose in
stimulating increased interest in vaccination, thereby helping to reduce the incidence of brucellosis in cattle to a point in many sections of the country where final eradication of the disease became a realistic and practical goal. During ensuing years the situation has changed materially from what it was in 1955. More than 90 percent of our bovine brucellosis now has been eliminated and increasing numbers of areas are either brucellosis free or are moving rapidly toward this status. Consequently, our procedures need to be more critical than ever before if the last reservoir of *Brucella* infection is to be identified and eliminated. While the dangers associated with titer tolerances in official vaccinates are no greater now than they were in 1955, they are assuming much greater significance as the goal of final eradication is approached. It is believed, therefore, that all interested groups should consider the advisability of eliminating the special titer interpretation granted official vaccinates.

For the past several years, my annual reports to this Committee have reflected strong optimism on the prospects of eradicating brucellosis from the entire country. On the basis of progress made each year, I am more convinced than ever that we have the knowledge and tools needed to reach this goal. It will be necessary, of course, to adjust procedures when necessary to meet changing conditions. Such changes have been made in the past and should be continued in the future whenever there is justification. The time required to attain final eradication will depend largely on the degree of cooperation and support provided by all interested groups. The 1965 goal is still a reasonable and realistic timetable.
REPORT OF THE COMMITTEE ON BRUCELLOSIS


Mr. President, Ladies & Gentlemen: Thomas A. Edison, the great inventor, was talking one day with the Governor of North Carolina, and the governor complimented him on his inventive genius.

"I am not a great inventor," said Edison.

"But you have over a thousand patents to your credit, haven't you?"

queried the Governor.

"Yes, but about the only invention I can really claim as absolutely original is the phonograph," was the reply.

"I'm afraid I don't understand what you mean," said the governor.

"Well," explained Edison, "I guess I'm an awfully good sponge. I absorb ideas from every source I can, and put them to practical use. Then I improve them until they become of some value. The ideas which I use are mostly the ideas of other people who can't develop them themselves."

Ladies and Gentlemen, this organization and its several standing committees are a great deal like "Edison's sponge." We meet each year in annual convention to absorb and gather ideas from every source possible and from these ideas we make recommendations that may be put into practical use in the field in the interests of animal disease eradication.

Your Brucellosis Committee has held hearings since Tuesday morning of this week. We have listened to research men, scientists, owners of livestock, in fact, every one who wished to be heard either for or against our present program insofar as it relates to the eradication of brucellosis in domestic animals. Research backed up by proven field trials and experience has given us all the tools necessary to complete the job of brucellosis eradication, which was started back in 1934. Those of us who are in a position to review the history of brucellosis eradication since 1934, will recall that no great progress was made until after World War II. Many of our states and the Federal Government as well, were in no position to continue the fight against this costly disease and to take part in an active and desperate world war at the same time. At the close of the war the demand for renewed activity in this field was very evident and in 1947 our present plan for carrying on the campaign to eradicate brucellosis
from domestic animals was re-inaugurated. Since that date great progress has been made, until at the present time, as reported to you by Doctor Mingle, we have 83 percent of the counties in the United States modified certified and 218 of these counties are not only modified certified areas but are certified free areas. With a very large percentage of the farm organizations, and livestock breeders cooperating, all states and the Federal Government working together have been able to make this record possible.

Tentatively, the year 1965 has been set as a date when all counties can report that they are in the modified certified area list, and it is realistic to expect that with reasonable cooperation from the areas that are not classified as certified, 1965 could well be the date when the entire United States and all counties within the United States could and we hope, will, be declared certified free.

Animal disease eradication is nothing new in the United States. We would call to your attention the fact that already contagious pleuropneumonia, foot and mouth disease, vesicular exanthema, glanders, dourine and fowl plague have all been eradicated, the last five during my lifetime. Pleuropneumonia was eradicated in the late 1880's and never since has raised its ugly head to make its appearance in these United States.

All states and the Federal Government are now engaged in an all-out program to eradicate brucellosis, tuberculosis, scabies, hog cholera, scrapie, and screwworms. Tuberculosis and brucellosis are in the final stages of this program. Both of these diseases are transmissible to human beings and therefore should receive priority attention. It wasn't so many years ago that thousands of hunchback children could be seen on our streets and in our communities. Today we see only a few such cases. The elimination of hunchback children can largely be accounted for because of the successful efforts in reducing the number of cattle with tuberculosis. The same thing holds true for human brucellosis or Undulant Fever, a disease with entirely different symptoms. Cases of the human form of this disease were once reported by the thousands, yet last year less than 500 cases were reported and human beings do not transmit the disease from one to another. Therefore, when brucellosis is completely eradicated from our domestic animals, there will be no more Undulant Fever in man. Our research people have told us time and again that cattle and swine having brucellosis are the primary sources of infection among human beings in the United States.

As Chairman of the Brucellosis Committee of the United States Livestock Sanitary Association and speaking for its Committee on Brucellosis, we want to congratulate every state official, every member of this organization and all organizations and individuals who have participated in the program to eradicate brucellosis. This program has been completed to a point where the final goal of complete eradication is now in sight, and we humbly ask that the representatives of those states and counties that do not find themselves in the certified area list, go home and do all within their power to help make this final run a complete success.
No program can stand still. We must plow ahead. With an agricultural industry representing three-fifths of the cash assets of all combined industries in the United States there is no place for a contagious or infectious disease among its domestic animals.

Your Committee has thoroughly reviewed the current uniform methods and rules as approved and published February, 1963, and it is our opinion that no changes are necessary at this time to effectively continue our present program. However, many requested subjects were thoroughly discussed and your Committee makes the following recommendations which we believe will materially aid the cooperative brucellosis eradication program.

1. Your Committee wishes to re-emphasize its endorsement of the swine brucellosis eradication program based on the validation of herds and areas, and urges all States to recognize validation as a basis for interstate movement of swine.

2. The Swine Brucellosis rapid card test is recognized by the committee as an alternate method for testing swine to achieve validation or re-validation. Your Committee recommends that this card test be maintained under the strict supervision of the Animal Disease Eradication Division, United States Department of Agriculture.

3. With more than 90 percent of the bovine brucellosis that once existed throughout the nation already eliminated and because of the growing need to minimize the dangers of infection being transmitted on occasion from official vaccinates, your Committee believes that all interested groups should consider the possible future need of eliminating titer tolerances now granted official vaccinates.

4. In the interest of minimizing the danger of persistent blood agglutination titers, your Committee urges that official vaccinations be applied as near the minimum recommended ages as possible (that is, four months).

5. At the 1962 meeting of this Association, a resolution was adopted concerning weaknesses of Part 78, Title 9 Code of Federal Regulations dealing with brucellosis. This resolution stated in part: "...the Federal Interstate regulations in its present form has served its original intended purpose and that it is now favoring the dissemination of the disease within certified areas and retarding the progress of the eradication program in non-certified areas." Special reference was made also to the dangers associated with specifically approved stockyards.

Resolutions of a similar nature have been received also from the National Brucellosis Committee, the North Central States Livestock Sanitary Association, and from individual States.

This matter has been studied further by the Brucellosis Committee here in Albuquerque and it is recommended that prompt action be taken by the Animal Disease Eradication Division of the United States Department of Agriculture to revise Part 78, dealing with brucellosis along the following lines:
(1) Consistent with sound disease control and eradication principles, maximum freedom of movement for cattle originating in certified areas should be provided.

(2) Cattle moving interstate should be qualified to move into any other area of the country including Federal Approved public stockyards, on the basis of the brucellosis status of the herds and areas of origin, and provided the animals involved are properly and permanently identified.

(3) It is suggested that the following classifications be used in qualifying animals for movements from their respective areas with progressively increasing restrictions being applied to classifications in the order listed:
   (a) Completely Certified State - maximum freedom of movement.
   (b) Non-Certified State with certified counties.
   (c) Certified free herds.
   (d) Herds in areas actively pursuing a program leading to certification.
   (e) Cattle from non-certified areas which originate in
      1) Blood test negative herds.
      2) Herds participating in the Market Cattle Testing Program and consistently negative.
      3) Herds consistently negative to the Brucellosis Ring Test.
   (f) Cattle of an unknown status (Identity has been lost).

6. Brucellosis in sheep does not constitute a problem in connection with the brucellosis eradication program in this country. There has been some indicated desire to employ Strain 19 vaccine along with ovis bacterin in controlling ram epididymitis. This procedure is currently being employed in New Zealand.

   The Committee believes that until further information has been developed with respect to the safety of vaccinating sheep with Strain 19, this procedure should be avoided. Also the Committee urges that studies designed to provide answers to this problem be carried out as soon as possible.

7. There are approximately 12,000 bison in the United States located in about 400 separate premises. Two-thirds of these animals have contact with cattle, either directly or across-fence. Half of the bison in the United States are located in 15 known infected herds. In view of the real hazards associated with these reservoirs of infection as related to the eventual eradication of brucellosis in the United States, your Committee believes that appropriate action should be taken to free the bison herds in this country of brucellosis as soon as possible. In this connection, the Committee endorses the proposed amendment to Part 78 of the Federal Interstate Regulation dealing with brucellosis as published in the Federal Register on July 18, 1963, and urges its early adoption.

8. The definition of an official vaccinate as listed in the uniform rules and regulations currently reads "Any female bovine animal"
vaccinated against Brucellosis with an approved Brucella Vaccine, etc., etc."

A request has been received by your Committee to amend the definition of "official vaccinate" to include the bull calf.

Your Committee spent considerable time discussing this request and it is of the unanimous opinion that with the information available it would be a dangerous procedure to recommend that bull calves be vaccinated with Strain 19.
A BOVINE FETAL VIRAL ISOLATE NEUTRALIZED BY IBR IMMUNE SERUM AS A CAUSE OF ABORTION IN CATTLE

G. N. Lukas, DVM; S. J. Weidenbach; K. G. Palmer, DVM; C. W. Dickie, DVM; R. F. Duncan, DVM; and J. Barrera, BA

During the period from February, 1961, to April, 1963 a cytopathogenic agent neutralized by infectious bovine rhinotracheitis immune serum was isolated from one hundred and fifty-two bovine fetuses representing abortion problems on twenty-eight premises. A review of the available literature did not disclose any report of isolation of the IBR virus from bovine fetal tissue. Several workers have associated this latter agent as a cause of abortion by serologic and clinical evidence. Because the IBR virus heretofore has not been presumptively incriminated as a causative factor in abortion, this report will include the results of the limited test animal inoculation trials to demonstrate pathogenicity and the role of the aforementioned cytopathogenic agent as a cause of rhinotracheitis, conjunctivitis, pustular vulvovaginitis and abortion in cattle.

The comparative studies of the fetal isolate were adapted from the reported isolation technics, cytopathogenicity, antibody response, cross-protection and reciprocal cross-serum virus neutralization tests, pathogenicity and intranuclear inclusion bodies of IBR virus. Both dairy and beef type fetuses were submitted for examination and the age and gross pathology were similar in each group. The clinical and field findings are to be reported. However, a brief description of the fetuses is deemed necessary to this article so there will be continuity of the findings as to age, type of tissue examined and the viral isolation results. The majority of fetuses were between six to eight months, and calves born alive were weak and died within a few days. Fetal isolates Nos. 2294, 2274, 2273, 2206 and 1697 were from separate herds with no IBR vaccination history.

Gross Description of Fetuses From Field Cases

Less Then Seven Months. Subcutaneous hemorrhage which was somewhat edematous and extended throughout the body, particularly in the umbilical, sternal and cervical regions; musculature of the abdominal, thoracic and cervical muscles were hemorrhagic as well as excessive sanguinous pleural and peritoneal fluid; perirenal hemorrhage and focal areas of hemorrhage in the kidney cortex and petechial to echymatic hemorrhages in the tracheal mucosa were the principle gross anatomic lesions.

From the Fresno Livestock and Poultry Pathology Laboratory, California State Department of Agriculture, Division of Animal Industry, Agricultural Veterinary Laboratory Services. Dr. G. N. Lukas, Veterinarian in Charge. Mrs. S. J. Weidenbach, Senior Technician, Virology Section. Drs. K. G. Palmer, C. W. Dickie, and R. F. Duncan, Veterinary Laboratory Diagnosticians. Mr. James Barrera, Senior Technician, Bacteriology Section.
Subepicardial hemorrhage rarely extended into the myocardium; the spleen was often enlarged four to six times and had a purplish hue, and the enlarged liver showed a characteristic dark, reddish-purple hue.

*Seven Months to Term.* There was progressive loss of the pathologic changes described in Group 1 as the fetus approached term. The enlarged spleen appeared to be the last tissue to regress to a normal size and many close to term appeared shrunken. The liver lost the congested appearance, was "doughy" in consistency and had a dull, reddish-yellow color. No perirenal hemorrhage and an absence of hemorrhages on the kidney surface and in the tracheal mucosa was common.

*Full Term.* The aborted full term calves or those born alive but weak and subsequently died within five days lacked any specific necropsy findings that could be described as consistent or pathognomonic. The spleen was sometimes half again its normal size but most often was found to be shrunken and pale. The liver was not enlarged and had a dull, yellow-orange-bronze streaked appearance and clay-like consistency. Usually the specimen was presented with the perianal area and tail soiled with a soft, pasty, yellow fecal material.

The above brief description is dissimilar to lesions observed in Epizootic Bovine Abortion (EBA) and therefore can be differentiated by the described anatomic lesions, isolation of a psittacosis-lymphogranuloma venereum (PLV) agent by yolk sac inoculation of six-day old chick embryos, and the histopathological findings. The characteristic pathologic changes observed in this latter infection includes edematous, gelatinous, subcutaneous tissues, particularly in the head region and a swollen, coarsely nodular liver. However, these changes are apparently secondary to the vascular lesions. The primary pathology, then, consists of petechial hemorrhages in the mucosa of the trachea, the tongue, thymus, salivary glands and the cranial lymph nodes. Small, gray foci are present in the ventricular myocardium and in the renal cortices. The fluid in the body cavities is usually straw colored and the ascites may produce a distended abdomen.

The nonsignificant and negative bacterial, PPLO, mycotic and leptospira findings prompted additional laboratory emphasis on the consistent recovery of the cytopathogenic isolate, subsequently to be reported. The characteristic bovine kidney cellular degeneration, the inhibition of the cytopathogenic effect by IBR immune serum, the correlation of the rise in neutralization titer of acute and convalescent sera from the dams, and the presence of intranuclear inclusion bodies warranted consideration that the CPA was identical or possessed common characteristics and antigens to the IBR virus.

**MATERIALS AND METHODS**

The routine diagnostic laboratory procedures on each fetus included cultures under reduced oxygen tension, carbon dioxide replacement, and aerobic as well as anaerobic atmosphere. Each tissue was processed on five and ten percent fresh sheep whole blood agar, thioglycollate, PPLO
broth and agar plates, and media for fungus isolation. Direct examination of body fluids for leptospira was done in addition to kidney and liver tissue sections. Yolk sac inoculation of spleen and liver was carried out on six-day old chick embryos. Bovine kidney mono-layer cell cultures were also inoculated with each of the fetal tissues and body fluids.

Every blood serum submitted by field veterinarians which was associated with an abortion problem was tested for *Brucella abortus*, *Leptospira pomona* and *Vibrio fetus* agglutinins, serum neutralizing antibodies of IBR and BVD (Bovine Virus Diarrhea), and carotene levels.

**Tissue Culture.** The established line cells of bovine kidney (MDBK) were provided by Dr. S. H. Madin and were maintained, transferred and cell cultures prepared by the described methods. The use of Modified Hank's solution with tris-buffer and lacto-albumin hydrolysate for transferring and establishing new growth and Modified Earle's low sugar balanced salt solution (Le) for maintenance has been entirely satisfactory. Initially, pooled lamb serum was obtained from a local packing house, however it has been replaced by a commercial source which had allowed for consistent duplication of results. Tubes and transfer bottles are seeded with approximately $1 \times 10^5$ cells per ml. The large volume of cell culture tubes used each week does not necessitate holding tubes for more than one week; therefore, it has been convenient to plan and prepare the following week's supply each Tuesday and Wednesday thus providing available cell cultures at their optimal growth.

**Control Virus and Serum.** The reference IBR virus and serum #321 was provided by Dr. D. G. McKercher. Laboratory isolates from field cases of rhinotracheitis, conjunctivitis, aborted fetal tissue and a commercial vaccine virus are also maintained for test. Respective immune sera were prepared by intranasal spray and intramuscular inoculation of susceptible cattle, and intramuscular inoculation of rabbits. The cattle received, by either route, five ml. of undiluted tissue culture fluid which had at least $10^7$ TCID$_{50}$ per 0.2 ml. and the rabbits were given two ml. of the same inoculum. The routine virus and serum neutralization tests were checked by cross-neutralization with a combination of the prepared antigens and immune sera. The virus stock was stored in a mechanical subzero refrigerator (-60°F) and the test virus is maintained and propagated by passage on MDBK cell cultures. The virus was harvested when the CPE approximated 75 percent of the cell sheet and the bottle was quick frozen and stored as the stock virus. The immune sera are stored at -20°F.

Regretfully, many of the early isolates have been lost primarily due to lack of proper refrigeration storage, however, the subsequent use of a CO$_2$ dry ice chest and mechanical subzero box (-60°F) enabled this laboratory to maintain 32 of the original 152 isolates. Five of the fetal cytopathogenic agents were used to prepare the control immune sera (#2294, #2206, #2372, #2373, and #1697). #2294 has been selected as the prototype of this group to present the comparative data with the reference IBR virus and immune serum. The following results will designate the latter virus and serum as IBR and IBR serum as compared to the fetal isolates and prepared immune sera by their respective numbers.
**Isolation Technique.** Approximately one gram of tissue was ground with Monterey sand and a 10 percent suspension prepared in physiological balanced salt solution (PBS) containing 1,000 units of penicillin, 0.5 mg. streptomycin sulfate and 0.005 mg. fungizone per ml. After thorough grinding of the tissues and the coarse pieces of tissues settled, the supernatant was quick-frozen at -60°F for at least 24 hours. The vials were thawed rapidly and a 1:5, 1:50, and 1:500 suspension in Le with three percent lamb serum was prepared for the preliminary virus isolation attempts. The growth media was poured off from the cell cultures and replaced with two ml. of each dilution of the suspect tissue after 48 hour incubation. Each culture tube was observed daily for CPE. The majority of the fetal tissues did not yield a significant concentration of the CPA at the first passage and often it was necessary to blind pass at least two to three times before the 5 x 10^{-2} cell tubes showed adequate SPE. Occasionally the first passage of inoculum from the placenta and umbilical tissues, umbilical veins and stomach contents would show excellent CPE at 5 x 10^{-1} dilution cell culture tubes. The cell cultures were incubated at 37°C for seven days. All cultures which did not demonstrate characteristic CPE were blind passed and the negative tissues and cultures were transferred four times before discarding. Prior to passage and subsequent titrations and neutralization of the CPA, the cell cultures were quick-frozen at -60°F for at least 24 hours then rapidly thawed in a 37°F water bath. A cell culture tube with a glass coverslip was included in each dilution providing a tube for reading the CPE and a monolayer of cells to be stained in hematoxylin-eosin and May-Gruenwald Giemsa stain and observed for intranuclear inclusion bodies. Cell cultures with CPE were further screened by preliminary titration of the CPA and virus neutralization tests at 1:5, 1:50, and 1:500 dilutions of this isolate using three cell culture tubes per dilution. If neutralization was observed, complete titration studies were conducted as described under virus titration and virus neutralization tests. Each fetal isolate was passed six times on MDBK cells prior to final virus titration neutralization and antibody response studies. A sterile, separate pipette was used for each dilution transfer in each procedure. In each of the following procedures the cell culture tubes were read and recorded after seven days incubation at 37°C with the exception of serum neutralization tests in which the CPE of the control virus tubes would be used as an indicator to determine the optimal time for the test to be read. Any degree of CPE was considered significant in calculating the endpoint.

**Virus Titration.** Virus titers were determined by preparing tenfold dilutions of the agent in maintenance fluid and 0.2 ml. of each dilution was inoculated into six cell culture tubes. The TCID₅₀ per 0.2 ml. was calculated by the method of Reed and Muench.

**Virus Neutralization.** Tenfold dilutions of the virus were prepared and alloquats of the virus dilutions and control serum (1:5 dil.) were mixed and incubated for 90 minutes at room temperature and 0.2 ml. of each mixture was added to six cell culture tubes per dilution.
Serum Neutralization Tests. Post-inoculation, acute and convalescent sera were used to test for neutralizing antibodies against IRB virus, and the fetal isolates. All sera were heat inactivated at 56°C for 30 minutes and then diluted 1:5 in Earle's maintenance fluid which contained a final concentration of three percent lamb serum. Serial twofold dilutions of each serum were mixed with the respective virus so that the serum/virus mixture contained 50 TCID\(_{50}\) of virus. The mixtures were incubated at room temperature for 90 minutes and two cc. of each dilution was transferred to each of three tubes of MDBK cell culture tubes.

Control Tests. Control series in the IBR virus and bovine fetal isolate cross-neutralization tests included a diluent control for each series of tubes, a virus titration without serum, a serum control of each serum sample (1:5 dil.) and a virus control containing the calculated TCID\(_{50}\) per 0.2 ml. of each virus.

RESULTS

Virus Isolations. Adopting the freeze-thaw technique to all tissues, including blood samples, increased the percentage of recovery to a relatively constant rate. The yield by direct inoculation of the coarse tissue suspension was somewhat indefinite as compared to the former method. The efficacy of this procedure was best illustrated by the negative isolation from blood samples prior to the use of the freezing method.

After the pattern of positive isolations presented in Table I was noted, greater effort was directed toward having the field veterinarians encourage the owner or herdsman to promptly obtain and submit the available placental tissues as well as the fetus. This procedure not only increased the opportunity of recovery but shortened the reporting time to the

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Positive Isolations*</th>
<th>Percent Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Placenta</td>
<td>53/53</td>
<td>100</td>
</tr>
<tr>
<td>2. Cotyledon</td>
<td>18/18</td>
<td>100</td>
</tr>
<tr>
<td>3. Umbilical Vein</td>
<td>12/12</td>
<td>100</td>
</tr>
<tr>
<td>4. Urachus</td>
<td>3/3</td>
<td>100</td>
</tr>
<tr>
<td>5. Liver</td>
<td>139/157</td>
<td>88</td>
</tr>
<tr>
<td>6. Spleen</td>
<td>130/157</td>
<td>83</td>
</tr>
<tr>
<td>7. Stomach Contents</td>
<td>7/12</td>
<td>58</td>
</tr>
<tr>
<td>8. Kidney</td>
<td>1/7</td>
<td>14</td>
</tr>
<tr>
<td>9. Trachea</td>
<td>3/42</td>
<td>7</td>
</tr>
<tr>
<td>10. Peritoneal Fluid</td>
<td>1/20</td>
<td>5</td>
</tr>
<tr>
<td>11. Lung</td>
<td>4/132</td>
<td>3</td>
</tr>
<tr>
<td>12. Pleural Fluid</td>
<td>3/106</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Number of isolations of the described fetal CPA over the total number of tissues cultured and the relative percentage of recovery.
veterinarian. In conjunction with recovery rate from various fetal tissues the range of virus concentration on first passage was: from placenta $10^{0.7} - 10^{2.7}$, cotyledons $10^{0.7} - 10^{2.1}$, umbilical $10^{0.7} - 10^{1.7}$, stomach contents $10^{0.7} - 10^{1.7}$, liver $0 - 10^{1.7}$, spleen $0 - 10^{1.7}$.

Total bovine isolations for the calendar year 1961-1962 reported as positive IBR virus or CPA neutralized by IBR immune serum from other than abortion problems are as follows: trachea 78, nasal washing 51, lung 48, liver 33, spleen 28, conjunctiva 19, brain 12, blood 18, milk two, semen one, and spinal fluid one. These findings illustrate the ubiquitous nature of the IBR virus and the described viral isolates.

**MBDK Cell Culture Cytopathogenic Effect.** The CPE of the IBR virus and prototype virus #2294 on MDBK cells were observed to be constant and the pattern of activity is identical (Figures 1 and 2). The intranuclear inclusion bodies shown in Figures 3 and 4 are the same respective fourth passaged viruses after 24 hours incubation stained by hematoxylin-eosin. Each caused a CPE from 24 hours to five days of incubation and there was consistently 75 to 100 percent degeneration of the monolayer on the fourth passage at 48 hours of incubation. This latter observation established the comparative virus titration studies to demonstrate virus concentration in tissue culture fluid at designated passages. Table II illustrates this.

![Figure 1. Cytopathogenic Effect of IBR Virus on Cell Strain of Bovine Kidney Line Cells (MDBK).](image1)

![Figure 2. Cytopathogenic Effect of Fresno Bovine Fetal Isolate 2294 on Cell Strain of Bovine Kidney Line Cells (MDBK).](image2)
Figure 3. Intranuclear Inclusion Bodies of IBR Virus in Cell Strain of Bovine Kidney Line Cells (MDBK) After 24 Hours Incubation. H-E Stain

Figure 4. Intranuclear Inclusion Bodies of Fresno Bovine Fetal Isolate 2294 in Cell Strain of Bovine Kidney Line Cells (MDBK) After 24 Hours Incubation. H-E Stain

TABLE II
Comparative Virus Concentration of IBR Virus and Bovine Fetal Isolates from Nasal Washing, Placenta, Umbilicus, and Fetal Liver After Repeat Passage

<table>
<thead>
<tr>
<th>Passage</th>
<th>Feed Lot Steer Nasal IBR Virus</th>
<th>Placenta</th>
<th>Fetal Isolates Umbilicus</th>
<th>Fetal Isolates Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.7</td>
<td>2.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>4.5</td>
<td>5.0</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>6.8</td>
<td>7.0</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>12</td>
<td>7.5</td>
<td>8.2</td>
<td>7.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*TCID 50 per 0.2 ml. of inoculum

comparison between an IBR virus isolated from nasal washing of a feedlot steer and three fetal isolates from placental, umbilical and liver tissue expressed as the logarithm of the dilution of virus.
Neutralization Tests. The results of the neutralization test of the IBR virus and #2294 isolate against IBR immune serum and immune sera prepared in susceptible cattle and rabbits with fetal isolates #2294, #2372, #2373, #2206 and #1697 graphically show in Table III the comparative CPE inhibition of the fetal isolate to IBR virus using 0.2 ml. of 1:10 ml. diluted serum mixed with 0.2 ml. of the respective virus dilution.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Log of Neutralized Virus</th>
<th>Rabbit Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log of Virus Titer</td>
<td>IBR 2294 1697 2206 2372 2373 Bovine Immune Sera BVD</td>
</tr>
<tr>
<td>IBR</td>
<td>7.0*</td>
<td>7.0 4.0 3.4 6.0 2.8 0</td>
</tr>
<tr>
<td>2294</td>
<td>5.0</td>
<td>5.0 3.8 4.0 4.0 3.4 3.4 0</td>
</tr>
<tr>
<td>1697</td>
<td>4.0</td>
<td>4.0 2.5 4.0 4.0 1.8 0</td>
</tr>
<tr>
<td>2206</td>
<td>5.0</td>
<td>5.0 3.4 4.0 4.0 3.4 3.4 0</td>
</tr>
<tr>
<td>2372</td>
<td>6.5</td>
<td>6.5 4.0 4.0 6.0 2.7 0</td>
</tr>
<tr>
<td>2373</td>
<td>5.5</td>
<td>5.5 4.0 3.4 5.0 3.0 0</td>
</tr>
</tbody>
</table>

*The figure is the logarithm of the virus dilution (TCID$_{50}$) after seven day incubation period.

This virus neutralization procedure was repeated with several hundred acute and convalescent sera representing respiratory or abortion problems submitted by field veterinarians. The results paralleled the neutralization titers shown in Table III. These studies also included BVD immune sera which served as a control serum. The pattern of inhibition against the described sera demonstrated the antigenic similarity of the six viral agents. The results of the serum neutralization studies in Tables IV and V showed a comparable correlation. Table IV is intended to show forty day post-inoculation antibody response in rabbits to the fetal isolates, and Table V the sera obtained from field outbreaks of rhinotracheitis, conjunctivitis and abortion in cattle. The antibody response in susceptible cattle by intranasal inoculation of Fresno isolate #2294 and IBR virus and the cross-reciprocal serum virus neutralization correlated the similarity of the test virus to IBR virus shown in the former comparative tests.

Reciprocal cross-serum/virus neutralization tests using #2294 isolate and the reference IBR virus as antigens were performed against serum from the cow from which fetal isolate #2294 was recovered and serum of cattle into which #2294 isolate had been inoculated. The results indicated that the cow from which fetal isolate #2294 was recovered had a significant increase in titer to both #2294 and IBR antigen and that there was reciprocal cross-neutralization between the two agents (Table VI).
TABLE IV
Comparison of IBR, BVD Virus and Bovine Fetal Isolates by Serum Neutralization Test

<table>
<thead>
<tr>
<th>Virus**</th>
<th>IBR</th>
<th>2294</th>
<th>1697</th>
<th>2206</th>
<th>2372</th>
<th>2373</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>2294</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>1697</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>2206</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>2372</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>2373</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>80</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>BVD</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>(Oregon)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reciprocal of dilution  
**50-100 TCID$_{50}$/0.2 ml.

TABLE V
Comparison of IBR Virus, Bovine Fetal Isolates, and BVD Virus by Convalescent Serum Neutralization Tests of Field Outbreaks in Cattle of Rhinotracheitis, Conjunctivitis and Abortion

<table>
<thead>
<tr>
<th>Virus</th>
<th>Serum Controls</th>
<th>Rhinotracheitis</th>
<th>Conjunctivitis</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IBR Immune</td>
<td>BVD Immune</td>
<td>Primary Feedlot</td>
<td>Feedlot and Dairy</td>
</tr>
<tr>
<td>IBR</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100*</td>
<td>20/20</td>
</tr>
<tr>
<td>2294</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100</td>
<td>20/20</td>
</tr>
<tr>
<td>1697</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100</td>
<td>20/20</td>
</tr>
<tr>
<td>2206</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100</td>
<td>20/20</td>
</tr>
<tr>
<td>2372</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100</td>
<td>20/20</td>
</tr>
<tr>
<td>2373</td>
<td>1/1</td>
<td>0/1</td>
<td>100/100</td>
<td>20/20</td>
</tr>
<tr>
<td>BVD</td>
<td>0/1</td>
<td>1/1</td>
<td>48/100</td>
<td>3/20</td>
</tr>
<tr>
<td>Oregon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numenator - Number of sera with neutralized antibodies  
Denominator - Number of serum tested

Transmission Trials

The selection of fetal isolate #2294 as the prototype of the group resulted primarily from the antibody response demonstrated by neutralizing antibodies and its cytopathogenic behavior on cultures of established bovine kidney line cells (MDBK). There was no attempt to screen the fetal isolates by their ability to cause severe respiratory symptoms.

These studies were designed to determine the pathogenicity of the isolate. In addition, the results of this trial will be correlated with the published information regarding the shedding period of this agent after infection, and the described cross-protection tests and antibody response for IBR and IBR-IPV viruses. 1,5,7,9,14,15,19,20,31,33,43,44,66
TABLE VI
Antibody Response of Cow to Fetal Isolate 2294 Demonstrated by Reciprocal Serum Neutralization Tests

<table>
<thead>
<tr>
<th>Virus**</th>
<th>Cow From Which Fetal Isolate 2294 Recovered</th>
<th>Calves 2294 Isolate Inoculated</th>
<th>Control IBR Cow Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days Post Abortion</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>IBR</td>
<td>1</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>2294</td>
<td>20</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>BVD (Oregon)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Reciprocal of dilution
**50–100 TCID₅₀ per 0.2 ml.

METHODS AND MATERIALS

Experimental Animals. Four pregnant Hereford heifers were obtained from a ranch that had been under study for two years prior to selection of the test animals. Over four hundred serum samples from this premise had been routinely tested for Brucella abortus, Leptospira pomona, and Vibrio fetus and carotene level determinations, and the results of the serum neutralization tests for antibodies of IBR and BVD were negative.

Each heifer was bled and swabs of the nasal, conjunctival and vulvo-vaginal mucosa were taken for pre-inoculation study. After pregnancy examination determined the viability and approximate age of the fetuses, the experimental animals were identified with the respective monthly age of the fetuses as heifer #1: 5-6, #2: 7-8, #3: 7-8, and #4: 8. These fetal age groups were preferred because they coincided to the age range of fetuses involved in field abortion problems.

Viruses. The infective agent was Fresno bovine fetal isolate #2294. This virus had been recovered from the liver of a six month fetus of the Hereford breed in October, 1962. The challenge respiratory IBR virus was a tracheal isolate from a feedlot steer. The reference IBR virus and immune serum had been provided by Dr. D. G. McKercher. Five ml. amounts of tissue culture fluid containing sixth passaged #2294 virus in screw cap vials was stored in the mechanical subzero freezer (-60°F). These vials were the source of inoculum for preparing the required amounts of virus for the subsequent tests and inoculations.

Routes of Inoculation. Inasmuch as the purpose of this trial was to establish whether the fetal isolate could cause abortion in cattle, each heifer was inoculated by the same routes. No contact or control animals
were used throughout the trial. It was hoped that the test heifers would act as the contact controls for transmission of the respiratory infection, and that each animal could provide the basis for cross protection tests with the challenge respiratory IBR virus.

Five ml. of undiluted cell culture fluid of sixth passaged 2294 virus containing $10 \times 10^7$ TCID$_{50}$ doses was injected intravenously. The same amount and dosage was instilled into the vagina of each heifer.

RESULTS

Clinical Response to Infection. The daily temperatures and clinical response are presented for each heifer on their respective charts (Figures 5, 6, 7, 8). Isolation attempts on vaginal, nasal, conjunctival exudates taken 72 hours post inoculation yielded a CPA neutralized by IBR and 2294 immune serum. The conjunctival, nasal, vulvo-vaginal mucosa and blood were cultured on MDBK cells prior to inoculation of 2294. Post inoculation cultures were taken on the third, 17th and 23rd day. The pre-inoculation as well as the 17th and 23rd day virus isolation attempts were negative. Each of the specimens taken on the third post inoculation day had a virus concentration exceeding $10^8$ on third passage. The data prior to the first passage could not be used because the initial cell cultures were positive at 1:5, 1:50 and 1:500 dilutions of the original specimens.

![Figure 5. Post-inoculation and Post-challenge Febrile and Clinical Response of Heifer #1.](image-url)
Figure 6. Post-inoculation and Post-challenge Febrile and Clinical Response of Heifer #2.

Figure 7. Post-inoculation and Post-challenge Febrile and Clinical Response of Heifer #3.
Therefore, it can be concluded that each sample contained a virus concentration exceeding $10^{2.7}$.

Serum neutralization tests to determine antibody response are recorded in Table VII and VIII and the reciprocal cross-neutralization studies are in Table IX. Because previous reports indicated no respiratory or ocular clinical responses to the intravenous inoculation of the IBR virus, it was assumed the nasal and conjunctival inflammation of the test animals resulted from contact exposure to the discharged vaginal inoculum. The febrile response of the test animals paralleled the findings of
TABLE VIII
Antibody Response to Heifers to Fetal Isolates 2294 Demonstrated by Reciprocal Cross Serum Neutralization Tests

<table>
<thead>
<tr>
<th>Virus</th>
<th>Serum—Cow 2294 Recovered from Fetal Liver</th>
<th>Antibody Titers*</th>
<th>Control IBR Cow Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum of 2294 Inoculated Heifers**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Numbers 1, 2, 3, 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days Post-Abortion</td>
<td>Days Post-Inoculation</td>
<td>Heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>IBR</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>2294</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>BVD (Oregon Strain)</td>
<td>Neg- Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Pre-inoculation neutralization titer negative against each virus.
**Reciprocal of Dilution.

TABLE IX
Cross Immunity Tests in Calves Between Infectious Rhinotracheitis Virus and 2294 Which Produced Infection in Cattle

<table>
<thead>
<tr>
<th>Infective Agent</th>
<th>2294</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>0/2**</td>
<td>0/2</td>
</tr>
<tr>
<td>2294</td>
<td>0/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

*Challenged 24 days post-inoculation
**Numerator = Number of cattle showing signs of illness
Denominator = Number of animals used

those reported for the experimental transmission of pustular vulvovaginitis,31 and exposure to IBR virus by the intranasal route.43 The temperature response of Heifer number one was similar to infection by intraocular inoculation reported in the same transmission studies.43 Each heifer showed some degree of hyperemia of the vulvar mucosa within 24 hours. In 48 hours a slight pustular vaginal discharge with small, focal, white pustules on the vulvar mucosa appeared. These pustules tended to form rows. A slight edematous swelling of the vulva was also present. In 72 hours some of the pustules coalesced producing a definite pustular vaginal discharge. Lacrimation, serous nasal discharge, anorexia, and listlessness were present at this time. The test animals were off feed to some degree for three to four days; lacrimation and a
nasal discharge were evident for six to nine days. The vulvar lesions gradually subsided by six to nine days. All animals were visibly normal within 13-14 days post inoculation.

As noted in Figure 5, heifer number one had a second elevation of temperature on the 11th-16th post inoculation days and aborted a six-month fetus on the 14th day. Heifer number three showed the most severe clinical response to infection, the vulvar lesions, pustular vaginal discharge, lacrimation, and nasal discharge were comparatively greater than the other three test heifers. On the eighth post inoculation day she became moribund but regained her feet and was eating within 24 hours. At this time she aborted an eighth month fetus. With the exception of heifer number four, which had an uneventful parturition and recovery, the other animals retained their placenta and had to be cleaned; however, the placental tissues from these latter heifers were removed easily with no complications.

Gross Description of Aborted Fetuses

Heifer #1. Estimated age of fetus was six months. The body was covered with fine hair; the hooves were soft, and the teeth had not as yet erupted. The primary necropsy lesion observed was the hemorrhagic subcutaneous edema extending from the throatlatch to the thoracic inlet; this decreased in severity along the ventral abdominal musculature.

The pleural cavity contained approximately 500 ml. of a post wine-colored fluid. The lungs were edematous. The heart displayed a few petechial hemorrhages of the epicardium extending into the myocardium. Primary abdominal lesions found were perirenal and subcapsular blood-colored edema. The spleen was enlarged approximately two to three times normal size. The liver had a doughy consistency. The remaining abdominal organs appeared blood stained, characteristic of fetuses that have died in utero.

A blood-tinged nasal discharge was present. There were, however, no hemorrhages in the trachea.

Heifer #2. Estimated age of this fetus was eight months. The teeth were barely erupted and had not yet calcified. The body was completely haired. Subcutaneous edema was absent. Musculature appeared pale. The prescapular lymph node was hemorrhagic.

50 cc. of blood-tinged fluid was present in the pleural cavity. A diffusely reddened pleura had no evidence of petechiation or other hemorrhages. There was approximately 25 cc. of blood-tinged fluid in the heart sac; in addition, approximately two-thirds of the surface of the myocardium displayed diffuse subepicardinal ecchymatic hemorrhages with scattered interspersed petechiae. The cut surface of the heart was somewhat bleached suggesting autolysis.

The abdominal cavity contained 50 cc. of straw-colored fluid. The liver was swollen and dull red in color. The spleen was approximately two times normal size. Petechiation was apparent in the perirenal tissue on the surface of the kidney. The other abdominal organs were grossly normal.
The fetal cotyledons showed evidence of necrosis.

Heifer #3. Estimated age of the fetus was eight months. The fetus was completely haired. The teeth were erupted. Localized petechiations were present on the rib cage, ventral abdominal area, and the hock area. The area over the thorax appeared dehydrated. The gelatinous, watery, subcutis frequently seen in many aborted fetuses was not present in this one. Muscular petechiation was minimal. Some were located adjacent to those seen in the subcutis. Diffuse petechiation was present throughout the trachea.

The 10 cc. of pleuritic fluid present was slightly blood tinged. Diffuse petechiation and some ecchymosis was present beneath the parietal pleura.

The thymus and heart had diffuse petechial and ecchymotic hemorrhages. A swollen, purplish liver, and a petechiated spleen of normal size were the only abnormalities seen in the abdominal cavity.

Heifer #4. This pregnancy resulted in a normal delivery of a live, term fetus. The calf was able to stand with much difficulty five to six hours after birth. It was observed nursing on two occasions. From the evening of the first day until it expired 36 hours later, it became progressively weaker.

The nostrils and muzzle were inflamed and there was peri orbital erythema. The teeth were calcified and the hooves were fairly solid suggesting the gestation period to be of normal duration or possibly ten days shortened. No gross abnormalities were observed in the skin or subcutaneous tissues other than a yellowish, gelatinous subcutaneous edema in the cariniform area of the sternum.

The trachea contained multiple ecchymotic hemorrhages throughout its course. The abnormalities of the thoracic cavity were limited to petechiae extending over one quarter of the pleural surface.

The only notable lesion in the abdominal cavity was the subcapsular petechiations of the liver. These hemorrhages extended down into the parenchyma.

A CPA demonstrating the same CPE on MDBK cells as IBR virus and the prototype isolate #2294 and which was neutralized by IBR and #2294 immune serum was isolated from each of the aborted fetuses. This agent was recovered from the following fetal tissues: umbilical tissue, stomach contents, liver, lung, spleen, body fluids (thoracic, peritoneal, pericardial), trachea, conjunctiva and the placental tissue. The highest concentration of the fetal isolate was recovered from the placenta, cotyledons, stomach contents and umbilical tissue. BVD immune serum did not neutralize any of the fetal isolates.

DISCUSSION

In the service area of this laboratory, IBR has been recognized as a serious clinical problem manifesting itself as the classical respiratory and ocular syndromes. Clinical observation and accepted laboratory procedures have established these syndromes in feedlot, beef and dairy herds. In addition to the isolation of the IBR virus from nasal washings and
conjunctival exudates, a viral isolate neutralized by IBR immune serum has been recovered from bovine fetal specimens, milk, vaginal discharge, preputial exudate, semen and a significant number of brains. These bovine viral isolates demonstrated the characteristics described for the virus of infectious bovine rhinotracheitis and as a cause of abortion by the use of the following criteria:

1. Tissue culture growth characteristics and cytologic degeneration.
2. Virus and serum neutralization tests.
3. Reciprocal cross-serum neutralization studies.
4. Antibody response.
5. Intranuclear inclusion bodies.
6. Pathogenicity studies.

Abortions have occurred in herds with histories such as (1) IBR-vaccinated and unvaccinated; (2) clinical evidence of upper respiratory signs; (3) ocular signs alone; (4) rhinitis alone; and (5) no clinical signs of IBR. An IBR virus recovered from an upper respiratory infection in cattle did not result in abortion when injected into pregnant cattle. These negative findings and field observations support the consideration that the fetal isolate, Fresno isolate #2294, which by comparison is characteristically similar to the IBR virus, may possess infective and antigenic properties not shared by the virus of IBR. Additional information enhancing this supposition is the reported erratic results of the commercially available IBR vaccines to stimulate adequate protection against abortion. These vaccines have been used prophylactically and during the onset of abortion.

Often, abortions have occurred three to five weeks after the introduction of new animals exhibiting conjunctivitis or rhinitis or other respiratory signs. In some of the latter herds, the upper respiratory infection did not appear to spread to the native cattle. The peak incidence of abortion was thirty to forty days after the first abortion. Sporadic losses then occurred for several months. Abortions usually occurred during the last four months of the gestation period.

While there were some cases of abortion in herds in which there were no clinical signs of IBR, the majority of abortions associated with the Fresno isolate have occurred in herds where classical signs of IBR have been observed.

This is in contra distinction to epizootic bovine abortion. In this disease, abortion is the sole clinical entity. Described histopathological findings, isolation of the PLV agent by yolk sac inoculation, as well as the lack of CPE on MDBK cells characterize the virus of EBA. Comparison of fetal anatomic lesions also provides differentiation of the two entities (Table X).

It is postulated that the fetal viral isolates characterized by Fresno #2294 will be proven to be IBR virus by biophysical means. The evaluation of the infective and antigenic components of the described fetal isolates may provide the means by which a vaccine for the prevention of IBR abortion can be produced.
### Table X
Comparative Gross Pathology Findings in EBA and Abortions Caused by Prototype 2294

<table>
<thead>
<tr>
<th></th>
<th>EBA</th>
<th>IRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Coarsely nodular, to absent</td>
<td>Swollen, darkened to clay-like consistency in older fetuses</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>Straw-colored</td>
<td>Sanguineous</td>
</tr>
<tr>
<td>Ascites fluid</td>
<td>Straw-colored</td>
<td>Sanguineous</td>
</tr>
<tr>
<td>Petechiation</td>
<td>Generalized</td>
<td>Localized and variable</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Petechiation, ecchymosis, wet, gelatinous</td>
<td>Sanguineous edema (5-7 month fetus)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Linear hemorrhages</td>
<td></td>
</tr>
<tr>
<td>Lymphoid tissue</td>
<td>Generalized enlargement</td>
<td>2nd trimester - hemorrhagic myositis</td>
</tr>
<tr>
<td>Kidney</td>
<td>Small gray foci (subcapsular)</td>
<td>with cooked appearance</td>
</tr>
<tr>
<td>Ventricular myocardium</td>
<td>Small gray foci</td>
<td>3rd trimester - lessening myositis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with cooked appearance</td>
</tr>
</tbody>
</table>

**SUMMARY**

The cytopathogenic isolates from bovine fetal tissue have been recovered by the methods proposed for the isolation of the virus of infectious bovine rhinotracheitis. The comparative serological studies by virus and serum neutralization tests, reciprocal cross-serum neutralization studies and antibody response demonstrated Fresno fetal viral isolate #2294 to be identical to IBR virus. In addition, the findings of this trial demonstrated that the Fresno isolate can cause infection resulting in abortion, pustular vulvovaginitis, rhinitis and conjunctivitis.

**ACKNOWLEDGEMENTS**

The authors are indebted to Dr. S. H. Madin for the initial instruction and encouragement to do tissue culture work; to Dr. W. W. Worcester for his support and suggestions; Dr. D. G. McKercher and Miss Midori Wada for their cooperation and appraisal of our early work; Drs. W. W. Watkins, C. S. Crane, R. S. Dickson and L. F. Ackermann for providing ample clinical material for study; and to the entire Fresno laboratory staff for their patience and response.

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Miss Midori Wada - Senior Technician, University of California, David, California
REFERENCES


IDENTIFICATION OF PNEUMOENTERITIS ISOLATES FROM CATTLE AS MYCOPLASMA*

Peter H. Langer** and Leland E. Carmichael***

In studies to determine the cause of calfhood illness characterized by pneumonia and enteritis, five cytopathogenic agents were isolated in cultures of bovine embryonic kidney cells from the blood and internal organs of dead or sacrificed calves. A sixth isolate was obtained from all quarters of the udder of a cow with bacteria-free mastitis whose calf died and showed pneumonia and enteritis.

Five of the six cytopathogenic isolates showed properties of limited filterability and hemagglutination (hemadsorption) of bovine and sheep erythrocytes. All strains were sensitive to ether and chloroform. These strains were not pathogenic for small laboratory animals, and showed limited growth in embryonating hens' eggs. Consistent growth was noted only in bovine and canine kidney cell cultures, as judged by cytopathogenicity and the hemadsorption phenomenon. Because of these properties and the failure to isolate bacteria on usual media, the isolates were regarded as viruses that shared certain characteristics with the Myxovirus group.

Further study of these isolates, however, revealed that hemadsorption and cytopathic effects were inhibited when certain antibiotics were added to cell culture media. Difficulties in effecting neutralization of the isolates by hyperimmune rabbit and calf sera also suggested that the behavior of these strains was unusual for viruses. Because of these observations, additional studies were made to identify and further characterize the isolates. Following culture of the original clinical specimens and early tissue culture passaged materials on various media designed for Mycoplasma, it soon became evident that all of the materials contained either Mycoplasma or mixtures of Mycoplasma and virus. Since uninoculated tissue cultures representing the original isolation media did not yield Mycoplasma, it is believed that the strains to be described represent agents present in the original materials reported earlier.

The purpose of this report is to correct the identity of the organisms reported earlier and to compare the properties of the isolated Mycoplasma

*This investigation has been aided by a grant from Nat. Inst. Health, U.S.P.H.S.

The authors are grateful to Dr. J. Fabricant and Dr. C. J. York for their suggestions.

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with those described for the "pneumonenteritis virus" isolates. In addition, evidence is presented to show that one of the isolates described originally contained a mixture of *Mycoplasma* and noncytopathogenic bovine virus diarrhea (BVD) virus.

**MATERIALS AND METHODS**

*Strains.* The source of isolates has been described in detail previously and strains are referred to in this report by their original identification number. The strains were designated as follows: 155, 531, 18, 527, 56R, and Sq. The first four strains listed were isolated from the blood and tissues of young calves and the last two were cultured from the milk and bronchial lymph node, respectively, of mature cows.

*Culture methods.* Methods of tissue culture were described previously. When it was found that all strains grew satisfactorily on a medium composed of beef heart infusion broth (Difco) supplemented with one percent yeast extract and 10 percent swine serum, this medium was employed for subsequent growth. Initially penicillin (1000 units per ml) and thallium acetate (0.1 percent) was added to all media to control bacterial contamination. For solid medium, beef heart infusion agar or PPLO agar (Difco) was used, supplemented with yeast extract and swine serum.

*Serological procedures.* Antisera were prepared against the various *Mycoplasma* strains in rabbits. A total of 50 ml of twice-washed eight-day *Mycoplasma* culture suspensions adjusted to an optical density (OD) of 1.0 at 530 microns was inoculated intravenously in graded doses over a period of 90 days, at which time satisfactory antibody levels were obtained. Then, animals were bled for serum that was employed for complement-fixation, agglutination, and growth inhibition tests. Also hemadsorption inhibition tests were performed with those strains that produced hemadsorption or hemagglutination (strains 155, 531, 18, Sq, and 56R). The procedures were described in the previous paper.

*Tests for BVD virus.* Tests for noncytopathogenic BVD virus in the original specimens were performed by the indirect method of induced cell resistance to a cytopathogenic BVD strain (C24V) as described by Gillespie et al. In addition, calves shown by serology to be susceptible to BVD virus were inoculated with certain of the original tissue culture passaged materials (strains 56R, Sq, and 155).

**RESULTS**

*Characteristics of the Mycoplasma strains.* Cultural examination showed that the six strains consisted of at least three distinct types as judged by colony morphology, rate of growth, carbohydrate fermentation patterns, and hemagglutination capacity (Table I). For all strains, five serial transfers of the original cultures on media that did not contain antibiotics or thallium acetate failed to result in the appearance of bacterial
<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Colony Forma</th>
<th>Colony Sizeb</th>
<th>Growthc</th>
<th>Reversiond</th>
<th>Hemagglutini</th>
<th>Reduction of Tetrazolium</th>
<th>Carbohydrate Fermentationf</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>spleen</td>
<td>1</td>
<td>0.08-0.14</td>
<td>R.T</td>
<td>neg</td>
<td>+ (1:32)</td>
<td>neg</td>
<td>±</td>
</tr>
<tr>
<td>56R</td>
<td>udder</td>
<td>1</td>
<td>0.09-0.15</td>
<td>R.T</td>
<td>neg</td>
<td>+ (1:32)</td>
<td>neg</td>
<td>±</td>
</tr>
<tr>
<td>531</td>
<td>lung</td>
<td>1</td>
<td>0.09-0.12</td>
<td>R.T</td>
<td>neg</td>
<td>+ (1:64)</td>
<td>neg</td>
<td>±</td>
</tr>
<tr>
<td>18</td>
<td>lung</td>
<td>1</td>
<td>0.08-0.14</td>
<td>R.T</td>
<td>neg</td>
<td>+ (1:64)</td>
<td>neg</td>
<td>±</td>
</tr>
<tr>
<td>527</td>
<td>blood</td>
<td>3</td>
<td>0.08±0.14</td>
<td>R</td>
<td>neg</td>
<td>-</td>
<td>neg</td>
<td>-</td>
</tr>
<tr>
<td>Sq</td>
<td>bronchial lymph nodes</td>
<td>2</td>
<td>0.02-0.04</td>
<td>R</td>
<td>neg</td>
<td>± (1:4)</td>
<td>neg</td>
<td>-</td>
</tr>
</tbody>
</table>

aColony Form 1—smooth, entire edge; opaque center; granular surface; uniform size
2—smooth, entire edge; no center; granular or coarse surface; uniform size
3—smooth, entire edge; faint center; granular surface; variable size

bSize measured after five days growth in serum broth, except for 527 which did not reach maximum until eight days.

cR means rapid growth in serum broth and colony formation in 24-48 hours. T means turbidity in serum broth in 72-96 hours.

dReversion to bacterial forms after five transfers in antibiotic-free media.

eHemagglutination of 0.25 percent sheep erythrocytes by five day broth cultures washed twice and adjusted to OD of 1.0 at 530 microns.

f± = partial change in phenol red indicator after four days
- = no change in indicator after seven days
forms. All strains were inhibited by the addition of tetracycline antibiotics (chlortetracycline, oxytetracycline and tetracycline) to the media at concentrations of 0.1 mg per ml and also by chloramphenicol and kanamycin (Kantrex, Bristol Laboratories) at concentrations of 0.06 percent. These antibiotics also inhibited cytopathic effects and formation of hemagglutinin in tissue culture by the original hemagglutinating strains. Only those *Mycoplasma* strains that previously had been considered hemagglutinating viruses showed hemagglutination and hemadsorption in tissue culture. One isolate (strain 527) that had failed previously to show hemadsorption in tissue culture also failed to show hemagglutinating activity when cultured in serum broth media.

None of the *Mycoplasma* strains grew in small laboratory animals or in embryonating hens' eggs. In broth culture, growth was strictly aerobic for all strains, however, growth on solid media required carbon dioxide, which was supplied by using a candle jar. None of the strains grew on cow or horse blood agar, a characteristic observed recently for certain *Mycoplasma* strains isolated from the bovine mammary gland. Litmus milk was not changed by any of the strains after five days incubation, nor was tetrazolium or methylene blue milk reduced.

Coverslip preparations of bovine kidney cells inoculated with two of the original "pneumoenteritis virus" suspensions (strains 56R and 155) and stained with Giemsa stain showed distinct coccobacillary forms ranging from 200 to 500 millimicrons in size. These were entirely within the cytoplasm of infected cells although extracellular forms were present. In severely infected cells, coarsely granular forms that resembled *Mycoplasma* colonies were observed in cytoplasmic vacuoles. These appeared to be amorphous eosinophilic masses when stained with hematoxylin and eosin and resembled virus inclusion bodies. Similar bodies were seen in the lungs of sick calves with the pneumoenteritis syndrome that yielded *Mycoplasma* (calves 155, 531 and 527). When stained with Gram's stain, the organisms were weakly Gram-negative.

**Serological comparisons.** Comparisons by reciprocal agglutination (Table II) and complement-fixation tests showed that all the strains were antigenically related. The results obtained by agglutination and complement-fixation tests were similar. There was no indication that these tests could differentiate between the *Mycoplasma* strains since all hyperimmune serums agglutinated the homologous and also the heterologous types, although other properties such as hemagglutination and colony morphology, noted above, suggested differences. By employing the hemadsorption-inhibition test, using as inoculum colony-selected *Mycoplasma* strains, the results were similar to those reported earlier.¹ There was complete serologic cross-reactivity between strains 56R, 155, 531 and 18, suggesting that these formed a single taxonomic group. Of the hemagglutinating strains, only Sq was unique.

Because the growth-inhibition test presently seems to be the most definitive one for the serotyping of *Mycoplasma*, this test was employed and used hyperimmune rabbit serum incorporated into serum agar at a concentration of four percent. Suppression or complete inhibition of
TABLE II
Serological Comparisons of Bovine *Mycoplasma* Strains by Agglutination Tests

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>155</th>
<th>56R</th>
<th>531</th>
<th>18</th>
<th>527</th>
<th>Sq</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>640</td>
<td>640</td>
<td>160</td>
<td>320</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>56R</td>
<td>160</td>
<td>NT</td>
<td>160</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>531</td>
<td>NT</td>
<td>160</td>
<td>160</td>
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<td>40</td>
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<td>80</td>
<td>160</td>
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<tr>
<td>527</td>
<td>40</td>
<td>40</td>
<td>NT</td>
<td>40</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Sq</td>
<td>160</td>
<td>320</td>
<td>NT</td>
<td>NT</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

*a*Titer values reported as reciprocal of serum dilution causing complete agglutination.

*b*All antigen suspensions adjusted to OD of 1.0 (650 microns).

Table:<br>
<table>
<thead>
<tr>
<th>Antiserum</th>
<th>155</th>
<th>56R</th>
<th>531</th>
<th>18</th>
<th>537</th>
<th>Sq</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>56R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>531</td>
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<td>±</td>
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<td>+</td>
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<td>527</td>
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<td>-</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Sq</td>
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</tr>
<tr>
<td>NRs</td>
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</tr>
</tbody>
</table>

+a* means complete inhibition of colony formation.

± means reduction in colony size and number of colonies, with involution forms.

- means no inhibition of colony formation.

*b*Normal rabbit serum (four percent) incorporated into agar media.

growth (colony formation) was taken to indicate antigenic similarity, although one strain (Sq) was not completely inhibited even by homologous serum. This strain (Sq) showed distinct involution forms when plated on agar containing homologous and also certain heterologous (strains 56R and 531) serums. Involution consisted of bizarre colony formation with vacuolization, stranding, and the assuming of amorphous shapes. The results of these tests (Table III) confirmed the findings reported previously, with the exception of strain 18 which now appears identical with 56R, 155 and 531.

Tests of isolates for the presence of noncytopathogenic BVD virus. In addition to *Mycoplasma* in the original specimens, the finding of BVD antibody in calves inoculated with strain 155 suggested the presence of BVD virus. A review of the pathological specimens from calf 155
Tests for Noncytopathogenic BVD Virus in Clinical Specimens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cell Resistance</th>
<th>Calf Serum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hyperimmune Rabbit Serum&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>56R</td>
<td>-</td>
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<tr>
<td>527</td>
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</tr>
<tr>
<td>Sq</td>
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</tbody>
</table>

<sup>a</sup>BVD-susceptible calves were inoculated intravenously with 5 ml of bovine cell culture fluid harvested five days after inoculation with suspensions of tissue from sick or dead calves.

<sup>b</sup>Hyperimmune rabbit sera used were those employed and reported in the original paper.¹

Also suggested this possibility since small erosions were observed along the edge of the tongue and numerous ecchymotic hemorrhages were seen on the mucosa of the rumen and the rugae of the abomasum. The presence of noncytopathogenic BVD virus was confirmed by the test for induced cell resistance to cytopathogenic BVD virus (Table IV), for strain 155 caused inhibition of cytopathogenic BVD strain C24V to a titer of 10<sup>3.5</sup>. Tests of the other five strains failed to show BVD in the original inocula. Tests of calves inoculated with strain 155 "pneumoenteritis virus" also showed a rise in BVD antibody. Calves inoculated with other strains failed to develop BVD antibody. Hyperimmune rabbit sera prepared with tissue culture fluid from the original isolates also showed BVD antibody only in serum prepared against strain 155. It, therefore, appears that the clinical disease observed in some calves in the earlier study resulted from a combination of agents.

Attempts to reproduce clinical illness with Mycoplasma. Inoculation of neonatal colostrum-deprived calves with the Mycoplasma strains (strains 155, 56R and Sq) selected from agar plates and grown in serum broth failed to produce significant illness when inoculated by various routes. Inoculated calves developed complement-fixing and hemagglutination-inhibiting antibodies that could be detected three weeks later, although growth-inhibiting antibody could not be demonstrated.

Broth cultures (72 hours) of strains 56R and 155 were instilled (0.01 ml) into two quarters, respectively, of the udders of two cows and acute, purulent mastitis occurred in the inoculated quarters within 72 hours. Spread to adjacent quarters was not observed. The cows recovered within 10 days and were subsequently immune to reinoculation to the homologous and also the heterologous strains.
DISCUSSION

This report has compared some of the biological characteristics of organisms previously described as "pneumoenteritis virus isolates" with those of *Mycoplasma* isolated from the original pathological specimens and stored tissue culture fluids. It was found that the properties of the isolates previously reported were consistent with those later observed with the various *Mycoplasma* types described herein, and it is, therefore, evident that the "virus isolates" should now be regarded as *Mycoplasma*.

The history of the role of *Mycoplasma* in pathogenic processes is documented by numerous instances where the etiologic nature and significance of isolates from pathological specimens had remained in doubt for years. For example, the well-known Eaton agent, first recognized as a cause of atypical pneumonia in man in 1944, was not identified until 1962 when Chanock *et al.* described its growth on artificial media and identified the agent as a species of *Mycoplasma*.

The six strains reported here appear to fit into three distinct types, based on colony morphology and serologic characterization by the growth-inhibition test. Such a grouping was indicated by the previous studies. Strains 155, 531, 18 and 56R seemed to form a single group. Strains Sq and 527, which showed weak hemagglutination and slight serologic cross-reactivity with 56R and 531, appeared to be separate types. These latter types differed also in growth characteristics from the other four strains, for Sq and 527 did not result in visible turbidity in serum broth until five to seven days, in contrast to the relatively rapid development of turbidity by the other strains.

A primary natural pathogenic role has been established only for one of the six strains reported here, 56R, which was isolated from the mammary secretions of a cow with purulent mastitis. The role of the other strains in bovine disease, especially calfhood illnesses, must remain presently uncertain since evidence thus far collected indicates that more than one agent was present in the tissues of some affected calves. Those strains of *Mycoplasma* thus far tested (56R, 155, 531 and Sq) did not produce significant illness when given alone. Final assessment of pathogenicity of these strains must await further work, especially when it is recalled that the organisms were initially isolated from calves that had recently received BVD virus or infectious bovine rhinotracheitis virus and which subsequently became ill with an intercurrent infection. The assessment of pathogenicity for *Mycoplasma*, with few notable exceptions, has been one of the most difficult laboratory problems, not only with mammalian but also with avian types. The isolation of hemagglutinating strains from pathological but not normal tissues and the presence of curious eosinophilic cytoplasmic inclusions (shown to be *Mycoplasma*) nevertheless suggests a pathogenic role for these agents in calfhood illness. As with other diseases where *Mycoplasma* assumes an important role in the pathologic process, for example, chronic respiratory disease of poultry, illness as seen in the field may not result unless *Mycoplasma* act in concert with other infectious agents or undefined inciting factors.
SUMMARY

Isolates previously reported to be viral agents associated with calfhood illness and bovine mastitis have been identified as *Mycoplasma*. The six original isolates were studied culturally and serologically and comparisons showed all *Mycoplasma* strains to be related by a common agglutinating antigen, but different by growth-inhibition tests and other biological characteristics. The six strains comprised three groups. Clinical specimens from one of the calves contained in addition to *Mycoplasma*, noncytopathogenic bovine virus diarrhea virus. A pathogenic role for these *Mycoplasma* strains in calfhood illness is suggestive but remains to be proved.

REFERENCES

Figure 1. Colony of strain 56R bovine *Mycoplasma* (5 day culture) photographed *in situ* (unfixed) on agar. 500X magnification.
Figure 2. Colonies of strain 527 bovine *Mycoplasma* photographed *in situ* (unfixed) on agar. Note two distinct colony forms. 500X magnification.
Figure 3. Colonies of strain Sq bovine *Mycoplasma* photographed *in situ* (unfixed) on agar. 500X magnification.
VIBRIOSIS IN RANGE CATTLE
Alvin B. Hoerlein, D.V.M., Ph.D.*
Fort Collins, Colorado

In 1958, A. H. Frank in a special report to this organization, stated that vibriosis was the most important single cause of bovine infertility. His statement was based mostly on experience in dairy herds and small farm herds of beef cattle. We would like to add our observations to his report and emphasize the most serious vibriosis situation recently found in Western range herds of beef cattle. Since detailed reports of our research carried out over the past four years are now in press, it seems appropriate for present purposes to digest some of our studies on vibriosis in range cattle and include only a minimum of specific data.

First, it should be pointed out that there is a wealth of knowledge concerning Vibriosis in cattle. Morrison's bibliography on *Vibrio fetus* lists 571 references to work published through 1959, most of which are concerned with the bovine disease. The pathogenesis and epizootiology are well understood. The commonly encountered misconceptions applied to bovine vibriosis are not the result of confusion in the minds of our mentors.

The essential features of the pathogenesis of bovine vibriosis can be briefly stated. This disease, caused by *V. fetus*, produces temporary infertility by causing early embryonal death. Aborted feti large enough to be observed are not common. The disease is spread almost exclusively by venereal means from infected cattle to non-infected cattle. All female cattle appear to be susceptible, but generally recover from the infection in two to nine months. While not all bulls are susceptible to vibriosis, those which contract the disease carry the infection for years. Some cows carry the infection over from one breeding season to the next without loss of fertility. Recovered female cattle have a definite resistance to the effects of the disease and reproduce normally even though bred to infected bulls. The semen from infected bulls may spread the infection when used artificially unless adequately treated to kill the bacteria.

Thus, the main signs of infection are those of poor conception; cattle returning to estrus after breeding, numerous non-pregnant cattle when examined for pregnancy, or a poor calf crop. When the infection is first introduced into the herd, all ages are equally affected, but after general spread through the herd, the losses are confined to replacement heifers. Due to the seasonal nature of breeding in range herds, calf crops of 20 to 50 percent are commonly observed following a breeding season of less than 90 days. Late calves are common if the breeding season is prolonged since cows recovering from the infection will conceive late in the breeding season.

*From the Department of Pathology and Microbiology, College of Veterinary Medicine, Colorado State University.*
season. The common ranch practice of breeding first calf heifers to young 
bulls with a low rate of infection, delays infection until their second breed-
ning season. While trichomoniasis is not being found, we should not forget 
that this disease has all of the clinical features of vibriosis.

Vibriosis appears to be more common in herds having improved 
breeding stock and has been found in all of the major breeds of beef cattle. 
Since most of the herds which we have investigated have been recently in-
fected, it has generally been easy to determine the probable point of intro-
duction of the disease. Purchase of bulls, especially from "dispersal and 
stock reduction sales"; expansion of the herd by purchase of cows or bred 
heifers; a wandering bull from a neighboring herd; borrowed or loaned 
bulls; and the purchase of a milk cow have been the sources most com-
monly found. Cattlemen from at least seven different states bought ani-
mals from one infected purebred herd during a "stock reduction sale." 
Ranchers often find open cows sold at public sales attractive since they 
can be purchased at slaughter prices. Vibriosis has been traced to pur-
chase of milk cows in at least two range herds.

Prior to activation of our research project, vibriosis had been 
diagnosed in our Diagnostic Laboratory by the bacteriologic examination 
of aborted feti. While most of these feti were from dairy herds, a few 
were from small beef herds in Eastern Colorado. Since abortions are not 
common, tentative diagnoses of vibriosis often had to wait a year or longer 
for confirmation. In our hands, serum agglutination and mucus agglutina-
tion were not satisfactory since both false positives and false negatives 
were commonly found. It is possible that our dissatisfaction with the 
mucus agglutination test stems from the fact that the stage of estrual 
cycle of beef cattle is generally not known.8

The application of the stainless steel apparatus of Frank and Bryner3 
for the collection of cervical mucus for bacteriologic examination proved 
to be a satisfactory, though cumbersome, method of confirming vibriosis 
in field herds. The simplified apparatus suggested by Seger13 provided an 
excellent method for the collection of cervical mucus and the transporta-
tion of these samples to the laboratory for culture. In a survey last fall, 
1301 cervical mucus samples were collected from non-pregnant cattle in 
83 beef cattle herds which were found to have less than ideal pregnancy 
rates when examined by practicing veterinarians. *V. fetus* was found in 
45 of these herds.7

Since these herds were selected because they had infertility problems, 
no estimation of the prevalence of vibriosis could be made, but it is im-
portant to note that infected herds were found in every geographic area 
studied throughout the state. However, we should quickly emphasize that 
Colorado is not at all unique in having vibriosis in range herds. We have 
made confirmed diagnoses of vibriosis in Wyoming, Nebraska, Kansas, 
New Mexico, and Texas. Numerous personal communications from veter-
inarians, regulatory officials, and ranchers from all of the western range 
states and provinces strongly suggest the presence of vibriosis in all 
range areas.
Unfortunately, control of vibriosis in range herds is most difficult. Exclusive artificial insemination is impracticable in most range herds. Artificial insemination followed by the use of clean-up bulls merely delays the time at which a cow will become infected and miss a pregnancy. The possibility of developing a non-infected herd from segregated virgin heifer and bull calves appears attractive. Our experience is limited to two herds. One herd, in which the non-infected cattle were isolated on another farm, has now gone for three years without infection in the young herd. In the other herd, having better than average ranch management, the non-infected herd was free of vibriosis for one year, but became infected the second. No error in isolation could be discovered. We believe that the long time necessary to replace the old infected herd makes perfect segregation too difficult to maintain. A single gate left open or fence jumped can spoil years of isolation in a day.

As a planned way to live with the disease, we recommend that year-old heifers be bred to the old infected herd bulls to expose them to infection at an opportune time. By use of a short (60 day) breeding period, the problem of late calvers is prevented. We expect a 20 to 30 percent calf drop from this breeding thus eliminating most of the problems of calving two-year-old heifers. When bred as two-year-olds, these heifers will be resistant to reinfection and with the added age and development should have a good calf crop. In large ranch operations having favorable weather and feed conditions, it has been profitable to have a second breeding season in late fall. A uniform crop of late calves can be marketed profitably and only a half year of production is lost.

Seeing no other likely method of satisfactory control of vibriosis under range conditions, we have concentrated on investigation of the resistance to reinfection present in recovered cattle. At this time our research appears to hold promise of the possibility of artificial immunization of cattle against the effects of vibriosis. In controlled exposure experiments we have found pregnancy rates of 80 to 100 percent requiring 1.2 to 1.8 breedings per conception in groups of heifers injected parenterally with living and dead \textit{V. fetus}. In untreated heifers (challenge controls) 10 percent pregnancy rates were found with 27 and 39 breedings per conception.\textsuperscript{4,5} Pregnancy maintained for 60 days has been the criterion of effectiveness used. Having thus demonstrated that the resistance of cattle can be increased by artificial means, we are hopeful that field tests now in progress may lead to a practicable method of vibriosis control in herds of range cattle. Much additional research can be foreseen to answer questions as to the possible influence on late abortions, carrier states, duration of immunity, antigenically different strains of \textit{V. fetus}, etc.

Our best recommendation at the present time is to ranchers having herds with no evidence of reproductive disease. Do not add any animals not \textit{positively} known to be virgin. Immature heifer and bull calves are the safest. This also and especially applies to dairy animals.
REFERENCES

REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


Mr. Chairman, Members of the Association, and Guests: In 1962, the Committee on Infectious Diseases of Cattle organized a Subcommittee to prepare a "Code of Minimum Standards for Health of Bulls and Hygiene of Bull Studs Producing Semen for Artificial Insemination" and a Subcommittee to make recommendations regarding "Contamination and Recontamination of Processed Fish, Poultry, and Animal By-products with Disease Producing Microorganisms."

The Subcommittee on Artificial Insemination prepared "Regulations for Bull Stud Health and Interstate and Intrastate Shipment of Semen." These regulations contained (1) "Suggested Model Regulation," and (2) a chart entitled "Uniform Certificate for Interstate and Intrastate Shipment of Bovine Semen for Artificial Insemination." The suggested Model Regulations and Uniform Certificate were adopted by the United States Livestock Sanitary Association. During this past year, these regulations have been found adequate and adopted by Montana and Virginia; thus uniformity in regulations, as recommended by this Committee and adopted by the United States Livestock Sanitary Association has been initiated.

It is the hope of your Committee that other states that feel the need for such regulations may follow this pattern in the interest of promoting uniformity in regulations and reporting forms for interstate and intrastate shipment of semen.

These certificates may be purchased from Dr. R. A. Hendershott, Sec.-Treas. of the United States Livestock Sanitary Association.

The Subcommittee on Contamination and Recontamination of Processed Fish, Poultry, and Animal By-products with Disease Producing Microorganisms prepared a sanitary code for processors.

After distribution of this code, processors of poultry and animal by-products recommended revisions suited to specific needs of their industry; and the processors of whole fish and fish material requested a separate code which would apply specifically to their needs. Their proposals were studied and an applicable code has been prepared for each industry.

This Committee is presenting a revised code on poultry and animal by-products entitled "Recommended Sanitation Guidelines for Processors of Poultry and Animal By-products," and a code for handling fish entitled, "Recommended Sanitation Guidelines for Processors of Whole Fish and Fish Material" with the recommendation that they be approved.

We wish to thank Dr. G. N. Lukas for his presentation "A Bovine Fetal Isolate as a Cause of Abortion" and Dr. A. B. Hoerline for his presentation "Vibriosis in Range Cattle." Both have been informative and of special interest at this time.

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I. INTRODUCTION

Poultry and Animal By-products are used in the manufacture of livestock and poultry feeds. As a result of many individual and cooperative studies, domestic and foreign reports have reported the contamination or recontamination of processed livestock and poultry by-products with *Salmonella* microorganisms. These studies indicate that this most likely occurs in handling and storage of the finished product.

II. PURPOSE OF THE RECOMMENDED SANITATION GUIDELINES

The purpose of this is to outline operating procedures to by-product processors for sound management and product handling in order to furnish a finished product that is free of *Salmonella*.

III. OFFICIAL RECOGNITION OF THESE SANITATION GUIDELINES

The by-product industry and/or allied utilization industries should consider giving special recognition to those by-product processors who operate under these guidelines.

IV. PLANT PREMISES

(The outline listed below may be used to accomplish the objective. This does not exclude the use of other equally effective procedures.)

A. GENERAL

1. Live animals and birds should not be allowed in "processed material" areas.
2. Rodent, vermin, bird, and insect control should be continually maintained in the plant.
3. It is preferred that no other business be conducted in the building. In the event that this is not practical avoid the use of equipment, tools, and personnel between businesses. Particular attention should be paid to protection of the "processed material" areas. "Processed material" areas should be kept clean from refuse, trash, or accumulation of "raw materials." All floor sweepings of the "processed material" areas should be reprocessed as raw material.

B. BUILDING CONSTRUCTION AND FACILITIES

1. Building construction and facilities vary from those of wide open construction in the deep South to those of completely closed masonry of steel construction in other sections of the country. The objective is to produce *PROCESSED MATERIAL THAT IS FREE OF SALMONELLA AND TO KEEP IT FREE*. The
building construction and facilities can be geared to fit each manufacturer's needs.

C. PROCESSING EQUIPMENT

1. "Raw material" should be heated to a temperature sufficient to kill all salmonella organisms. Temperature reading through the use of a recording thermometer should be representative of the entire lot of material being processed. This can be accomplished by making certain that the material is continually agitated during processing.

D. EMPLOYEE FACILITIES

1. Adequate personnel showering, dressing, and disinfecting facilities should be available for employee use.

E. BY-PRODUCT PROCESSING AND STORAGE AREAS

1. The processing area of a plant should be divided into "processed material" area and "raw material" area. These areas should be separated by a solid, non-leaking floor or wall.

2. If different personnel are not available for each type of area, those personnel utilized in both areas should use specially marked outer clothing, gloves, head, and footwear in each area. It should be routine that personnel wash their hands thoroughly with soap and water before going into the "processed material" area from the "raw material" area.

3. Equipment such as shovels, brooms, etc., used in the "processed material" areas should be specially marked and used only in the "processed material" areas.

F. GENERAL RECOMMENDATION ON STORAGE

1. There should be continued effort to minimize the storage time of both raw and processed animal by-products. Cutting down storage time of both raw and processed products lessens the chances for cross-contamination of harmful germs from one part of the plant to another.

2. It is preferred that personnel assigned to the "raw material" area should not be used in the "processed material" area and vice versa.

G. MOISTURE CONTROL

1. Storage areas, walls, floors, and ceilings should be leakproof to keep out moisture.

2. The processed product should be kept dry at all times. The use of storage bins rather than putting processed product on the floor will lessen the possibility of its being seeded with Salmonella or of its getting wet. Germs require moisture to multiply so the finished products or containers or areas where it is stored should be kept dry to keep germ numbers down.
H. DUST CONTROL

1. Continuous attention should be given to controlling and removing accumulated processed product dust which settles on shelves, window sills, equipment, etc. This may be accomplished best by vacuuming. Do not use water or steam for this because it will serve to establish pockets or pools of damp processed materials in which germs may grow.

I. FACILITIES TO SCRUB OFF FOOTWEAR

1. Containers and scrub brushes and a disinfecting solution should be placed outside all entry ways into a "processed material" area. The footwear of all personnel should be scrubbed off using the solution and a stiff brush before entering the area. This procedure is considered one of the most important in the effort to control Salmonella organisms.

V. VEHICLES

A. TRUCKS, OTHER TRANSPORTATION VEHICLES, AND CONTAINERS USED FOR HAULING "RAW MATERIAL"

1. Truck-beds, tanks, and barrels used for transporting raw material by-products should be leakproof and so constructed that no drippings or seepage can escape.

2. Collection vehicles used for transporting dead stock or any product of dead stock should be thoroughly scrubbed out and disinfected before hauling each load.

B. TRUCKS USED FOR HAULING "PROCESSED MATERIAL"

1. Trucks or other vehicles used for transporting processed bulk or packaged animal by-product material should be thoroughly cleaned and disinfected prior to hauling each load.

VI. CONTAINERS FOR "PROCESSED MATERIAL"

Only new or sterilized used containers should be used for packaging processed by-product materials. Containers should be stored in such a manner that they do not become contaminated.

VII. LABORATORY EXAMINATION

A. There should be laboratory examination of "processed material" for the presence of Salmonella organisms to assure that these above procedures are effective.

B. A composite sample should consist of eight one-ounce portions from each shipment. (1) If the shipment is sacked or packaged in similar containers, half ounce (one tablespoonful) samples should be obtained from sixteen containers to make a 95 percent probability composite sample of eight ounces. (2) If the shipment is bulk, a core sampling rod can be used to collect a composite sample of the "processed material" totaling eight ounces per shipment. (3) Ice
cream containers or strong paper sacks, properly identified, make excellent composite sample containers.

C. Each shipment of "processed material" should be sampled and saved for a period of sixty days. This is to assure that any future question of the presence of *Salmonella* in a shipment can be checked against the "processed material" sample.

D. Samples being held "on the shelf" during the sixty day period should be subjected to random laboratory examination as determined by an appropriate authority.

See USDA Handbook (ARS 91-36) "Recommended Procedure for the Laboratory Isolation of *Salmonella* Organisms from Animal Feeds and Meat By-Products" for laboratory culture techniques for isolating *Salmonella* organisms.

**VIII. TRAINING**

All plant employees should be thoroughly trained in Plant Sanitation Guidelines and the need for strict adherence to an accepted set of procedures in order to obtain a product that is free of *Salmonella*.

**IX. GENERAL APPLICATION**

In applying the foregoing procedures, by-product processors should strive in general for maintenance of forward product movement with adequate precautions during storage and shipping in order to prevent post-processing contamination from in plant and outside sources.

**X. RESPONSIBILITY FOR SANITATION GUIDELINE COMPLIANCE**

Key plant personnel should be trained as a security or safety officer to ascertain that all aspects of the guidelines are carried out. If processed products are found to be contaminated through laboratory examination, the sanitation procedures of the plant should be re-examined and corrective measures instituted.

**XI. PLANT SANITATION**

Cleaning agents are applied in hot-water solution with a stiff brush or other suitable apparatus in order to remove grease and all other material sticking to the surface of the object being cleaned. A good detergent should be used.

Generous applications of flowing hot water and brisk scrubbing along with and following the cleaning operation will assure the most satisfactory results. All surfaces should be free of grease and accumulated material following the scrubbing and cleaning operation prior to applying any disinfecting solution.

**XII. DISINFECTANTS**

After a thorough physical cleaning, a disinfectant having recognized germicidal properties should be used to aid in the destruction of any remaining organisms.
XIII. GLOSSARY

A. ANIMAL BY-PRODUCT

For the purpose of these procedures animal by-product means livestock, poultry, or any of their products.

B. CLEANING AGENTS

Agents useful in removing grease and/or other material sticking to the object being cleaned.

C. "PROCESSED MATERIAL" AREA

Area used for manufacturing, transporting, handling, or storing the processed animal by-products.

D. "RAW MATERIAL" AREA

Area used for transporting, handling, or storing non-processed animal by-products.

E. RAW MATERIAL

Animal by-product that has not been properly processed.

F. PROCESSED MATERIAL

Animal by-product that has been heat treated sufficiently to cause fractionation under manufacturing procedure.

G. SALMONELLA CLEAN BY-PRODUCT

"Processed material" in which the presence of Salmonella is not detectable when sampled by procedures outlined in this guideline and subjected to laboratory examination as described in USDA Handbook (ARS 91-36).

H. DISINFECTANT

Agent that destroys infective germs when it is applied in an appropriate manner.
RECOMMENDED SANITATION GUIDELINES FOR PROCESSORS OF WHOLE FISH AND FISH MATERIAL

Recommendations concerned specifically with the genus *Salmonella*

I. INTRODUCTION

Whole fish and fresh raw or cooked fish material from filleting or cannery lines are used in the manufacture of fish meal for use in livestock and poultry feeds. As a result of individual and cooperative studies, domestic and foreign reports have indicated the possible contamination or recontamination of the dried fish scrap and meal with *Salmonella* type microorganisms. These studies indicate that this most likely occurs in handling and storage of the finished product.

II. PURPOSE OF THE RECOMMENDED SANITATION GUIDELINES

The purpose of these guidelines is to outline operating procedures to fish meal processors for sound management and product handling in order to furnish a finished product *that is free of Salmonella*.

III. PLANT PREMISES

The guidelines listed below may be used to accomplish the objective. This does not exclude the use of other equally effective procedures.

A. GENERAL

1. Live animals and birds should not be allowed in "processed material" areas.
2. Rodent, vermin, bird, and insect control should be continually maintained in the plant.
3. No other business should be conducted in the buildings used for handling, processing, or storage of raw fish material or the processed product. "Processed material" areas should be kept clean and free from refuse, trash, discarded and broken machinery, and semi-processed material such as press cake.

B. BUILDING CONSTRUCTION AND FACILITIES

1. Building construction and facilities vary from those of wide open construction in the deep South to those of completely closed masonry or steel construction in other sections of the country. The objective is to produce *PROCESSED MATERIAL THAT IS FREE OF SALMONELLA AND KEEP IT FREE.* The building construction and facilities can be geared to fit each manufacturer's needs.

C. PROCESSING EQUIPMENT

1. Control procedures should be initiated to assure that the raw material has been heated to a temperature sufficient to *kill all Salmonella organisms*. 
D. FISH MATERIAL PROCESSING AND STORAGE AREAS

1. The processing area of a plant should be divided into "processed material" areas and "raw material" areas. These areas should be in separate buildings or otherwise effectively separated.

2. Insofar as possible, different personnel should be used in each area. Where personnel must work in both areas, use of protective caps, dust coats, and shoe covering for use only in the scrap curing and storage areas should be considered.

3. Cleaning equipment (brooms, etc.) also shovels and tractor-driven scrapers used in moving the piles of scrap should not be used outside the scrap curing and storage area. When this area includes several buildings, paved, hard-surfaced walk or driveways that can be kept clean and dry should connect buildings. If practical these passageways should be covered.

E. MOISTURE CONTROL

1. Storage areas, walls, floors, and ceilings should be leakproof to keep out moisture.

2. The processed product should be kept dry at all times. Germs require moisture to multiply so that a dry scrap curing and storage area helps keep germ life of all types, including Salmonella, at low levels.

F. DUST CONTROL

1. In the curing process for the dried fish scrap employing the dropping method of aerating and cooling, the small particles of the material that collect on the floor in the vicinity of the scrap pile should be cleaned up when the dropping procedure is discontinued. After the fish scraps have been moved out and the shed is empty, it should undergo a cleaning process for removal of residues of scrap and dust before receiving new material.

G. PREVENTION OF CONTAMINATION BY FOOTWEAR

1. All entryways to "processed material" areas should be protected by shallow pans containing a pad or mat wet with disinfectant. The pan should be the length of the entry and wide enough so as not to be easily stepped across. Maintenance is important. Pads should not be allowed to dry out or become filled with dirt, and a stable disinfectant of adequate strength should be used in the pan. The footwear of all personnel entering these areas should be scrubbed off with a disinfectant solution, using a stiff brush. Containers of disinfectant and brushes should be kept outside all entryways. This precaution is considered very important in efforts to control Salmonella.
IV. TRANSPORTATION

A. VESSELS AND TRUCKS FOR TRANSPORTING "RAW MATERIAL"

1. Whole fish are normally brought directly to the plant in the catching vessel. Vessel holds should be thoroughly washed down with clean water after each trip.
2. Trucks, tanks, or barrels used to hold and transport fish material from processing lines to fish meal plants should be of tight leak-proof construction.

B. TRUCKS, FREIGHT CARS, AND BARGES FOR TRANSPORTING "PROCESSED MATERIAL"

1. The equipment used for transporting the "processed material" is a serious potential source for contaminating a "Salmonella-clean product" as shipped. Frequently this equipment is not the property of the shipper, and thus there are acknowledged difficulties in maintaining it in clean condition. Notwithstanding, all equipment should be inspected before loading to see that it has been properly cleaned. If it is not cleaned, it should receive proper cleaning. All carriers should cooperate in this requirement.

V. CONTAINERS FOR "PROCESSED MATERIAL"

Only new or sterilized used bags or other containers should be used for packaging "processed material." Empty bags should be stored in such a manner that they do not become contaminated before use.

VI. LABORATORY EXAMINATION

A. There should be a periodic laboratory examination (ARS 91-36) of "processed material" for the presence of Salmonella organisms to assure that these procedures are effective.

See USDA Handbook (ARS 91-36) "Recommended Procedure for the Laboratory Isolation of Salmonella Organisms from Animal Feeds and Meat By-Products" for laboratory culture techniques for isolating Salmonella organisms.

B. A composite sample should consist of eight one-ounce portions from each shipment. (1) If the shipment is sacked or packaged in similar containers, half-ounce (one tablespoonful) samples should be obtained from sixteen containers to make a 95 percent probability composite sample of eight ounces. (2) If the shipment is bulk, a core sampling rod can be used to collect a composite sample of the "processed material" totaling eight ounces per shipment. (3) Ice cream containers or strong paper sacks, properly identified, make excellent composite sample containers.

C. Each shipment of "processed material" should be sampled and saved for a period of sixty days. This is to assure that any future
future question of the presence of *Salmonella* in a shipment can be checked against the "processed material" sample.

**VII. TRAINING**

A. All plant employees should be thoroughly trained in these Plant Sanitation Guidelines and in the need for strict adherence to them.

**VIII. RESPONSIBILITY FOR SANITATION GUIDELINE COMPLIANCE**

A. Key plant personnel should be trained as a security or safety officer to ascertain that all aspects of the guidelines are carried out. If processed products are found to be contaminated through laboratory examination, the sanitation procedures of the plant should be reexamined and corrective measures instituted.

**IX. CLEANING AGENTS**

Cleaning agents preferably are applied in hot water solution. Any good commercial detergent can be used. A steam gun or high pressure jet of detergent solution should be used to loosen and remove grease and other material sticking to the surfaces being cleaned. Surfaces should be free of grease and adhering material following the cleaning operation prior to applying the disinfecting solutions.

**X. DISINFECTANTS**

After a thorough physical cleaning, a disinfectant having recognized germicidal properties should be used to aid in the destruction of any remaining organisms.

**XI. GLOSSARY**

A. *FISH MATERIAL*

Fish material means whole raw fish, raw wastes such as remain after removal of fish fillets or cooked waste.

B. *CLEANING AGENTS*

Commercial detergents useful in removing grease and/or other material sticking to the object being cleaned.

C. "*PROCESSED MATERIAL*" AREA

Area sued for curing, turning, storage, grinding, and loading of dried scrap or meal.

D. "*RAW MATERIAL*" AREA

Area used for unloading, measuring, transporting, handling, or storing raw fish or non-processed fish material.

E. *RAW MATERIAL*

Fish material that has not been properly processed.
F. PROCESSED MATERIAL

Fish material that has been heat treated during processing sufficiently to reduce moisture content to less than 10 percent.

G. SALMONELLA-CLEAN FISH MATERIAL

"Processed material" in which the presence of Salmonella is not detectable when sampled by procedures outlined in this guideline and subjected to laboratory examination as described in USDA Handbook (ARS 91-36).

H. DISINFECTANT

Agent that destroys infective germs when it is applied in an appropriate manner.
EXPERIMENTAL RINDERPEST IN SHEEP

T. L. Barber, D.V.M., M.S., and W. P. Heuschele, D.V.M.*

From ancient times, rinderpest has been well known as a devastating plague of cattle. In outbreaks among fully susceptible cattle, morbidity approaches 100 percent and mortality may exceed 90 percent.

The disease in sheep is much less striking. In 1922,9 sheep were used to obtain a rinderpest virus for vaccine which would be free of cattle blood parasites. Sheep became infected when inoculated subcutaneously or dosed orally with viremic blood from rinderpest-infected cattle. Signs of illness in sheep were inconspicuous and included only a rise in body temperature and a mucous discharge from the nose. Blood taken from these sheep six days after inoculation was infective for cattle, whereas urine and feces taken simultaneously were not. Sheep did not become infected by contact. This observation was later confirmed and it was observed that cattle in contact with infected sheep did not become infected.10 Another worker5 found that blood from infected sheep was infective for cattle on the ninth, but not on the thirteenth day after inoculation. Contact transmission was observed from infected sheep to normal sheep. Sheep infected with either of two virulent strains of rinderpest virus had an inconstant and slight temperature rise. These findings and a trial using a caprinized strain of rinderpest virus7 indicated a mild clinical response of sheep to experimental infection with rinderpest virus.

An excellent documentation of a laboratory-confirmed outbreak of rinderpest in sheep has appeared2 which would seem to contradict these observations. The outbreak began in cattle and in one week spread to sheep in contact. Within 11 days after the first were observed to be infected, 65 of 110 sheep had clinical signs and 13 died. All the sheep not dying during this period were slaughtered. Clinical signs and necropsy lesions in sheep were very similar to those seen in cattle. Diarrhea and intestinal lesions as severe as those of infected cattle were observed. The diagnosis was confirmed by a complement-fixation procedure with tissues from both cattle and sheep.

The purpose of this study was to determine the effect of a virulent strain of rinderpest virus on sheep raised in the United States and to study clinical signs of illness and methods of diagnosis.

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*Present address: East African Veterinary Research Organization, Kikuyu, Kenya, East Africa.

This is to gratefully acknowledge the services of Dr. R. P. Lehmann, Biometrician, Livestock Research Staff, Biometrical Services, Agricultural Research Service, USDA, Beltsville, Md., who did the statistical analyses of the data. The technical assistance of M. E. Hiddink and P. Mikiciuk is also acknowledged.
Inoculum.—Infection was initiated and immunity was challenged with rinderpest virus (Pendik). The virus was inoculated subcutaneously in a one-ml. dose known to contain over 10,000 bovine lethal doses.

Diagnostic tests.—1. Virus neutralization test. Serums were assayed in rabbits by the method of Stone and DeLay. All were tested in pools from two or three animals grouped according to days after inoculation and age except lamb 822 which was also tested individually.

2. Complement-fixation test. Serums and tissues were tested individually, using the method of Moulton and Stone.

3. Agar gel diffusion. The agar double-diffusion technique used was the method of White as modified by Stone and DeLay.

4. Subinoculation of cattle. Sheep were bled six to 10 ml. from the jugular vein with EDTA (Disodium salt of ethylene diamine tetraacetic acid) as an anticoagulant. Blood samples from sheep were pooled and immediately inoculated subcutaneously into one steer.

Clinical determinations.—Temperatures were recorded daily and sheep were examined for signs of illness. For hematological determinations in trial one, five ml. of blood was taken using EDTA as an anticoagulant. Complete blood counts included: total red and white and differential white cell counts, erythrocyte sedimentation rate (one hour), hemoglobin determination, and packed red cell and buffy coat volumes. Methods used in hematologic determinations were described previously.

Experimental animals.—All sheep were of mixed breeds. In trial 1, eight ewes, two-three years old, and eight lambs, six months old, were used. In trial 2, six sheep, one-eight years old, were used. The cattle were 1-1/2- to two-year-old grade Herefords. Rabbits were adult New Zealand whites.

Experimental procedure.—The procedure for carrying out the trials was as follows:

Trial 1.—Ten sheep, five ewes and five lambs, were inoculated with rinderpest virus after temperatures and complete blood counts were recorded for three days. Of these, two (814 and 824) were killed at six days postinoculation (DPI) and two (816 and 826) at 13 DPI for necropsy. The immunity of the remaining six (815, 817, 818, 822, 823, 825) was challenged at 21 DPI, and they were killed 26 days later. Complete blood counts were made through 14 DPI and three-seven days after challenge inoculation.

Controls, three lambs and three ewes, were held in two additional rooms. Complete blood counts and temperatures were recorded, the same as for the principals. One lamb and one ewe (819 and 593) were inoculated with a bovine tissue suspension comparable to the virus inoculum. The immunity of these two was challenged at 21 DPI, and they were killed 35 days later.

Two control animals were killed with the first two principals as normal necropsy controls. Two sheep (664 and 821) were used as normal controls for temperatures and complete blood counts and were later utilized in one attempt at infection by contact. The lamb and ewe were
placed in a room with a heifer that was subsequently inoculated with Pendik strain rinderpest virus. The heifer was removed on the third day of fever and the sheep were given challenge inoculation of rinderpest virus nine days later. Responses to exposure and challenge were measured by determination of body temperature and total white blood cells per cu. mm.

Trial 2.—Six sheep were inoculated with rinderpest virus. Two each were killed on three, five, and 10 DPI. Whole blood was taken for subinoculation into cattle from all six at three DPI and from those remaining at five and 10 DPI. Spleen and mesenteric lymph nodes from each animal were frozen and held for serological studies. Total white blood cell counts were done on all animals alive at 0, three, five, seven, and 10 DPI and temperatures were taken daily.

Statistical analysis.—Eight inoculated sheep in trial 1 were held 13 days or longer. Temperature and blood count measurements from these animals, with their controls, were treated statistically. The method of least squares with individual regressions to the fifth power was used in order to obtain the relatively straightforward curves in Figures 1-4. In each figure, the curves represent four inoculated ewes, four inoculated lambs, two control ewes, and two control lambs.

RESULTS

Trial 1.—Neither lambs nor ewes were severely affected by the rinderpest virus. Transitory inappetence and slight nasal discharge were of insufficient severity to be of diagnostic value. The temperature responses of these animals are shown in Figure 1. The highest temperature curve represents four inoculated lambs. The curve representing the two control lambs is significantly lower (P < .01). Both groups of ewes had rectal temperatures lower than the comparable group of lambs; there was a significant difference (P < .01) between inoculated and control ewes.

![Figure 1](image-url)  
*Figure 1. Temperatures of four ewes and four lambs inoculated with rinderpest virus and of two normal ewes and two normal lambs.*
Figure 2. Total white blood cell counts of four ewes and four lambs inoculated with rinderpest virus and of two normal ewes and two normal lambs.

Figure 3. Lymphocyte totals of four ewes and four lambs inoculated with rinderpest virus and of two normal ewes and two normal lambs.
Figure 4. Neutrophil totals of four ewes and four lambs inoculated with rinderpest virus and of two normal ewes and two normal lambs.

Figure 2 shows the total white blood cell count results for the same groups of animals. The leukocyte count was reduced from the third to the twelfth day in inoculated ewes and lambs. One of the four lambs (822) failed to have a lowered white count below a one-day low of 6,100 WBC/cu. mm. 10 DPI. This animal had a temperature response (peak 105.4 C), developed complement-fixing antibodies by 13 DPI, and was not affected by challenge. Lymphocytes in differential white cell counts, in the same groups of animals (Figure 3), were lowest in the inoculates from two to eight DPI with a mild secondary reduction around 12 DPI. The neutrophils (Figure 4) were reduced on eight to 11 DPI. There was no shift to the left associated with the neutrophil drop. Monocytes, eosinophils, and basophils remained relatively stable in the differential counts.

Erythrocyte sedimentation rate, hemoglobin, total red cells, and packed red cell and buffy coat volumes were not significantly altered during the infection.

### TABLE I

<table>
<thead>
<tr>
<th>Animal</th>
<th>Before inoculation</th>
<th>13 days post inoculation</th>
<th>21 days post inoculation</th>
<th>14 days post challenge</th>
<th>21 days post challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb 815</td>
<td>0</td>
<td>1:80*</td>
<td>1:160</td>
<td>1:160</td>
<td>1:160</td>
</tr>
<tr>
<td>Lamb 817</td>
<td>0</td>
<td>1:80</td>
<td>1:80</td>
<td>1:80</td>
<td>1:80</td>
</tr>
<tr>
<td>Lamb 818</td>
<td>0</td>
<td>1:80</td>
<td>1:160</td>
<td>1:160</td>
<td>1:160</td>
</tr>
<tr>
<td>Lamb 822</td>
<td>0</td>
<td>1:160</td>
<td>1:320</td>
<td>1:80</td>
<td>1:320</td>
</tr>
<tr>
<td>Ewe 823</td>
<td>0</td>
<td>1:40</td>
<td>1:320</td>
<td>1:160</td>
<td>1:160</td>
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<tr>
<td>Ewe 825</td>
<td>0</td>
<td>1:160</td>
<td>1:320</td>
<td>1:160</td>
<td>1:320</td>
</tr>
<tr>
<td>Lamb 819</td>
<td>0</td>
<td>0</td>
<td>1:320</td>
<td>1:160</td>
<td>1:80</td>
</tr>
<tr>
<td>Ewe 593</td>
<td>0</td>
<td>0</td>
<td>1:80</td>
<td>1:160</td>
<td>1:80</td>
</tr>
</tbody>
</table>

*Highest dilution of serum giving 50 percent fixation of complement.*
TABLE II

Results of Rinderpest Virus Neutralization Test in Rabbits with Sheep Serums

<table>
<thead>
<tr>
<th></th>
<th>Final virus dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>Nakamura III lapinized rinderpest virus</td>
<td>2/2*</td>
</tr>
<tr>
<td>Virus with rinderpest-immune rabbit serum</td>
<td>2/2</td>
</tr>
<tr>
<td>Pool of ewe 593 and lamb 819:**</td>
<td></td>
</tr>
<tr>
<td>13 days after normal tissue inoculation</td>
<td>2/2</td>
</tr>
<tr>
<td>14 days after virus challenge</td>
<td>2/2</td>
</tr>
<tr>
<td>35 days after virus challenge</td>
<td>2/2</td>
</tr>
<tr>
<td>Pool of lambs 822, 818, 817, 815:**</td>
<td></td>
</tr>
<tr>
<td>13 days postinoculation serum</td>
<td>2/2</td>
</tr>
<tr>
<td>21 days postinoculation serum</td>
<td>2/2</td>
</tr>
<tr>
<td>21 days post challenge</td>
<td>2/2</td>
</tr>
<tr>
<td>Pool of ewes 823 and 825:**</td>
<td></td>
</tr>
<tr>
<td>13 days postinoculation serum</td>
<td>1/1</td>
</tr>
<tr>
<td>21 days postinoculation serum</td>
<td>2/2</td>
</tr>
<tr>
<td>21 days post challenge</td>
<td>2/2</td>
</tr>
<tr>
<td>Pool of ewes 816 and 826:**</td>
<td></td>
</tr>
<tr>
<td>13 days postinoculation serum</td>
<td>2/2</td>
</tr>
<tr>
<td>Pool of ewe 664 and lamb 821:**</td>
<td></td>
</tr>
<tr>
<td>14 days post exposure</td>
<td>2/2</td>
</tr>
<tr>
<td>14 days post challenge</td>
<td>2/2</td>
</tr>
<tr>
<td>Serum of lamb 822:**</td>
<td></td>
</tr>
<tr>
<td>21 days postinoculation serum</td>
<td>2/2</td>
</tr>
<tr>
<td>**Preinoculation serum</td>
<td>2/2</td>
</tr>
</tbody>
</table>

* Rabbits infected/inoculated.

Complement-fixation (CF) tests (Table I) indicated that antibody was present in inoculated sheep as early as 13 DPI and in most cases was increased by 21 DPI. The virus neutralization test in rabbits (Table II) gave similar results. The six inoculated sheep remaining were not affected by the challenge virus at 21 DPI.

The lamb and the ewe inoculated with noninfected bovine tissue comparable to the virus inoculum did not become febrile, had no leukopenia and developed no rinderpest antibodies as measured by complement fixation and neutralization tests. They were used as challenge virus controls at 21 DPI, whereupon they responded with a leukopenia and elevated temperature. They were held longer (35 days) after inoculation with rinderpest virus than other sheep. At 35 DPI, their complement-fixing antibody
level was declining (Table I), whereas their neutralizing antibody had not decreased from its 14 DPI level. The two sheep in contact with a rinderpest-infected heifer, from day of inoculation through the first three days of fever, remained normal. When their immunity was challenged two weeks later, their response included a temperature rise and a leukopenia.

**Trial 2.**—Viremia tests were positive through 10 DPI. Cattle, inoculated with blood taken from sheep at three, five, and 10 DPI, died at six, six, and eight DPI, respectively, with typical signs of rinderpest infection.

Virus was detected by complement fixation in only one of the six sheep. Spleen and mesenteric lymph node extracts fixed complement in the presence of hyperimmune rabbit serum in dilutions of 1:40 and 1:75, respectively. These tissues were from one of two animals killed five days after inoculation. Agar gel diffusion tests were negative when spleen and mesenteric lymph nodes were tested from all six animals. The leukopenia and temperature elevation was similar to that observed in ewes in Trial 1. No gross lesions were found at necropsy of animals in either trial which could be attributed to rinderpest.

**DISCUSSION**

Results of this study confirm other laboratory trials involving rinderpest in sheep and contribute information on blood cellular changes during the infection. Neither this nor the previous laboratory trials help to explain the report of an outbreak of rinderpest in sheep in which the disease resembles classical rinderpest of cattle. This, and other laboratory trials, indicate that experimental rinderpest in sheep is a mild and clinically inapparent syndrome. Nevertheless, since neither all strains of the virus nor all breeds of sheep in stress situations have been included in laboratory trials, a more severe infection may be encountered in the field. Accidental importation of this disease in other than the usual host, especially a host wherein the disease may be mild and clinically inapparent, may be more likely than importation in cattle.

The most acceptable diagnostic methods currently available for rinderpest in sheep are the subinoculation of acute phase blood into susceptible cattle or the detection of antibodies in convalescent serum either by the virus neutralization test in rabbits or by complement fixation. Leukopenia was a reliable indication of laboratory-induced infection. The leukopenia was of longer duration and was more severe than the temperature elevation. However, the failure of one lamb to develop a definite leukopenia indicates that this criterion alone is not an adequate guide to experimental infection. If the disease produces a more severe syndrome in the field, complement fixation and agar gel diffusion tests using sheep tissues deserve further consideration for help in diagnosis.
SUMMARY

Sheep were experimentally infected with a bovine-lethal strain of rinderpest virus. The clinical response consisted of an elevated temperature during the third through seventh days after inoculation and a reduced total white blood cell count beginning on the second and persisting through the twelfth day after inoculation. Signs of illness were not obvious and gross lesions were not found.

REFERENCES

Despite the advances in many countries of the world—specifically in veterinary sciences' contribution to increased food production—a great many areas are not only failing to keep pace but are actually regressing in terms of keeping up with population requirements.

Re-orientation in disease control philosophy has created a new concept of preventive rather than curative veterinary medicine and this, in turn, has resulted in an international and regional approach to the disease control problems of the world. In short, the futility of unilateral disease efforts in respect to epizootics has been demonstrated and the success of numerous recent campaigns have proved the value of concerted effort.

It is not alone the efforts to control diseases of livestock that have contributed to an ever increasing animal protein food supply, but also the efforts in husbandry and management. Still these combined efforts fall short of the ever increasing demands for better nutrition for an explosively expanding population. Innumerable problems—emerging diseases, vector expansion and callous neglect of precautionary measures—conspire to thwart the gains achieved in disease control in localized areas. And these are the areas in which The Scope of International Veterinary Work can be appreciated.

The remainder of this presentation is a verbatim reproduction from the last part of Dr. Cockrill's paper.

* * * * *

FAO is one of the 13 Specialized Agencies of the United Nations. It has its own budget in the form of funds committed by its 104 member nations, by far the greater majority of which are primarily recipient rather than donor countries. Its headquarters are in Rome, and, at the present time, due to an increase in its normal operating funds, and to participation on a large scale in the operations of the United Nations Special Fund and the FAO-sponsored Freedom from Hunger Campaign, the Organization is going through a phase of rapid development and expansion.

*The paper presented here is, in part, an abstract, and in part, the text of a paper delivered by W. Ross Cockrill at the University of Cambridge on February 27, 1963. It is a concise, yet descriptive, account of the problems confronting veterinarians and the significance related to the solution of these problems.
We have about 850 experts in the field, of whom 65 are veterinarians. These veterinarians are working in 35 countries and are engaged in almost every conceivable field in which a veterinarian can operate. In every case, their work is directed towards two ends—the increase of food supplies, not only in a specific country, but for world consumption; and the control of diseases intercommunicable between animals and man (and these are usually of an economic as well as of a public health importance).

FAO, of course, is not by any means the only body operating internationally in the field of animal health. It is appropriate here to mention the great efforts made by certain of the Metropolitan powers over a period of many years. It is, perhaps, one of the world tragedies of this age that so much careful, painstaking work over so many years, by so many dedicated foreign service officials is being swept away by the tidal waves of nationalism. Fortunately, in many countries, the services built up under colonial systems still remain, and are likely to be further strengthened as stability is achieved. On these foundations much of the work of the Agencies is based and, indeed, for some years past the former Colonial Services have been a fruitful source of recruitment of the Agencies. For rather obvious reasons this source is now a dwindling one.

International cooperation in animal disease control is also sponsored by the World Health Organization, which has a primary interest in the zoonoses. The work of FAO and WHO in this and related fields is closely integrated, and a high measure of cooperation exists between the two Agencies. Their functions in promoting the interchange of technical information, in stimulating research and survey work, in initiating international collaboration and, above all, in providing a wide variety of technical assistance, are perhaps not as widely known and appreciated as they ought to be.

L'Office International des Epizooties, with its headquarters in Paris, was established as long ago as 1924 as an intergovernmental body. It has a unique reputation as clearing house for the collection and dissemination of information on livestock diseases. It publishes many bulletins and is concerned with the elaboration of advice on control methods.

These are three permanent bodies concerned with animal disease control on an international scale. There are many other organizations in existence which incorporate this aim in their operations. Of these it is fitting that special mention be made of the World Veterinary Association, which celebrates its centenary this year. This is a truly international body concerned exclusively with veterinary science.

The famous "Point Four" programme of the United States Government, and the wide-ranging Colombo Plan, under the umbrella of bilateral assistance agreements, have provided outstanding guidance and assistance to many countries in the field of animal disease control.

Many of the technically advanced countries are not coming forward with their own projects for bilateral assistance to developing areas. A significant trend in such forms of aid is that the advice and support of FAO are being increasingly sought by both the donor and the recipient countries, and in some cases this type of aid is actually being channelled
through FAO, thus utilizing the Organization's extensive knowledge and experience, and its established machinery of technical assistance to underdeveloped territories.

THE VETERINARY EXPERT

The Latter-day Pioneers

The quality of an organization like FAO is assessed in many countries by the quality of its experts. The term "expert" is used because it means approximately the same thing in all the official languages, and is not open to the misinterpretation which sometimes arises over terms like "specialist" or "consultant." Unfortunately, it lays the individual open to a certain amount of facetious comment, and an expert has been variously and unkindly defined as "anyone who is working in a country other than his own," or "someone who is sent out to find out, and gets out before he is found out."

The job is invariably difficult and sometimes dangerous; it is always demanding, it is often excessively frustrating, it is conducted under living conditions which are usually far from being of a high standard of comfort, and it makes great demands on the professional skill, tact and staying power of the individual. It is, perhaps, a measure of the success we have achieved in the general field of technical assistance to the underdeveloped countries, that we never have sufficient funds to meet all the requests which are made to us for the services of experts.

Field Veterinary Activities

It may be that special assistance is sought in the control of one particular disease. For example, in some African and Far Eastern countries FAO experts have played, and are playing, an important part in bringing rinderpest under control. Technical assistance of varying kinds provided by FAO has had a considerable part in controlling the disease, in limiting spread, and even in eradicating it completely in some areas.

In the control of rinderpest, and increasingly in other diseases, stress is laid on the use of living attenuated virus-vaccines. These frequently have to be produced in the countries in which they will be used, and FAO specialists may be required to set up laboratories, bring them to a state of operational efficiency, and train local personnel in the specialized techniques involved. Following field tests and surveys decisions have to be made on the most suitable types of vaccine for the particular circumstances.

Nowadays, our member countries are looking to us more and more to provide emergency assistance when epizootics appear. Recently we have been deeply concerned with the alarming epizootic of African horse sickness throughout the Near East, an example of disease introduction and spread through a susceptible animal population which demonstrated how very tenuous our control can be. In this particular case we provided expert assistance in the production of vaccines, undertook the training of
specialists from the affected countries, donated equipment and supplies and coordinated the national efforts in Saudi Arabia, Syria, Iran, Iraq, Turkey, Afghanistan, India and Pakistan in one of the worst epizootics in 10 years. It is beginning to appear as if those measures were effective and, if there is no flare-up this year, the countries will be able to compliment themselves on the success of one of the biggest campaigns of its kind ever attempted.

FAO, subject always to the limitations of funds, supplies the necessary laboratory equipment, for example freeze-drying apparatus, without which the scope of control programmes would be severely limited. Local personnel have to be advised on all the intricate details of vaccine production, and the maintenance of complicated modern equipment. Plans have to be made for the most efficient use of vaccination teams and arrangements have to be made and put into practice for the proper storage, handling and transport of vaccine. Local veterinarians may have to be advised on the details of operating large-scale vaccination programmes, and lay vaccinators have to be properly trained and supervised. Mobility is essential: poor communications and lack of transport are limiting factors to many assignments. Any philanthropic body which will present us with anything up to 100 Land Rovers, equipped as Mobile veterinary dispensaries, will make a great contribution to development.

Of the more insidious diseases, which extract an incalculable toll annually, parasitism is probably the most notable. It is undoubtedly one of the heaviest brakes on progress in livestock production. FAO veterinarians, specially selected for their knowledge of modern methods of diagnosis, prophylaxis, treatment and control of parasitism are in demand by our member countries all over the world. Great strides are being made in all aspects of parasitology. The application of new techniques, some of them very cheap and simple, can yield spectacular results in improved production and the prevention of losses, occasionally in a very short space of time. Until relatively recently this was a Cinderella branch of veterinary science.

In almost all the countries in which FAO veterinarians operate, advice and assistance on the control of tuberculosis, brucellosis, and mastitis are considered an essential part of the duties of the assignment. Much the same applies to anthrax and blackleg, which are receiving more attention as countries realize that losses which have been accepted as inevitable for generations can be prevented by vaccines produced by modern techniques.

Diseases transmitted by vectors such as ticks and biting insects are well recognized as highly important causes of reduced productivity and heavy mortality in immense areas of the world. Governments are showing a growing interest in this problem and technical assistance is being made available on an increasing scale by FAO. We have established an Expert Panel on Tickborne Diseases, which is comprised of the foremost specialists in this field and which—following the pattern of such bodies—meets at intervals to discuss the latest advances, to coordinate research and to issue reports on the most efficient and up-to-date means of control. Apart from its direct value to the FAO veterinarian who is specializing in this
field, the reports of the Panel are proving to be of very great importance to the large number of countries where such diseases are not only a cause of extensive financial loss but, as in the case of trypanosomiasis, are among the greatest impediments to progress, expansion and the welfare of essentially agricultural communities.

Adequate control of the various groups of diseases depends, in a very large degree, on control of the vectors, and especially on improved means of preventing their transport from country to country, and continent to continent, by such media as the ubiquitous and highly suspect aircraft. This is a factor of increasing importance in the international approach to disease control. In many cases, for example, African horse sickness, the full extent of vector involvement is not yet known, nor is the life-cycle always understood. Until research on such matters is greatly extended and strengthened, the control of vectors will continue to be inadequate. In such extremes as the tsetse areas of Africa, millions of square miles of territory will remain virtually closed to livestock production until cheap and effective insecticides can be produced and applied in quantity, or other methods such as biological control can be utilized for the eradication of vectors, and hence for the control of livestock diseases.

Some FAO veterinary assignments have unusual or even spectacular aspects: one of our experts, for example, is concerned with the Auchenidae, that rather mysterious group of animals which includes llamas and alpacas, the husbandry and health of which has been largely neglected, although they are of major importance in Peru where there are some 4,000,000 of them. Another FAO veterinarian is engaged in the control of disease, principally parasitism, in working elephants. Several are deeply concerned with the domestic buffalo, of which there are about 80,000,000 in the territory stretching from Egypt to Vietnam. This is an animal which science has neglected, presumably because, for the most part, it exists only in under-developed territories. It is, however, of immense importance, and whole systems of agriculture, such as the rice-producing areas, are entirely dependent on the health and work capacity of the buffalo.

A Yugoslav veterinarian is working for FAO in the field of the zoonoses and veterinary preventive medicine in the South American region. A Danish FAO veterinarian is assisting the Central American Republics in quarantine matters. A British veterinarian—formerly with the Ministry of Agriculture—is helping to control a bad outbreak of foot-and-mouth disease in Ecuador. A former R.A.V.C. officer is assisting the Indian Government in their forceful campaign to eradicate rinderpest. A Turkish FAO expert is working with the veterinary department of Afghanistan. A Texan, who was formerly with FAO Mission in Paraguay and subsequently took the Diploma in Tropical Veterinary Medicine at Edinburgh, is now in charge of the FAO rinderpest control team in Cambodia. A Swiss veterinarian is helping the Government of Thailand with the operations of the only foot-and-mouth disease laboratory in the Far East. A British veterinarian is working at the new veterinary school in Burma and is doubling that task with the difficult job of FAO mission chief. A U.S. FAO expert, on safari in the Chaco of Central South America, was instrumental in
saving the lives of 10 Indians who had eaten anthrax-affected meat. With no medical services within several hundred miles, he pumped all his available supply of penicillin into them. An Indian veterinarian is introducing artificial insemination into Syria. A Japanese is working on virology in the near East.

I cannot say that the list is endless. Unfortunately we have many fewer veterinarians in the field than are required.

*Animal Reproduction*

FAO veterinarians have pioneered the use of artificial insemination in many parts of the world, and now that new techniques are being discovered and widely applied, we are assisting countries in their proper use and application. Artificial insemination, properly used, is an important technique in the field of animal reproduction. Wrongly or carelessly used, it can do incalculable harm to breeding programmes, and to breed improvement. In FAO, we are constantly concerned that all advances, such as the deep-frozen semen techniques, should be used sparingly in the less-developed countries until their dangers and drawbacks, as well as their advantages are fully appreciated. We have learned through long experience that, of all the assignments in which FAO veterinarians are engaged, those concerned with artificial insemination can be the most difficult and sometimes the most dangerous. We arrange, wherever possible, for follow-up action to such assignments to ensure that the expert's recommendations are being followed, and we try from headquarters to keep in touch with the responsible technicians in the countries concerned. In this field, also, the responsibilities of the veterinarian are very great and, as his activities increase in the general field of livestock production and improvement, those responsibilities become heavier.

*International Veterinary Careers*

International work in veterinary medicine provides I think, one of the most interesting and rewarding fields which the profession has to offer. It is little wonder that we are inundated with inquiries as to how to enter this branch of the profession. It is sad to have to record the fact that 84 percent of all applications have to be rejected, generally, I am sorry to say, on the grounds of youth and inexperience. Our member countries will seldom accept the services of any other than senior and experienced veterinarians. Almost without exception, therefore, we can only recruit men with a minimum of 10 years' practical experience, and a definite field of specialization.

Until quite recently international work has offered very little opportunity for a continuing career. The tendency was to recruit high-level men for brief periods of up to one year to carry out an assignment in a specific field. The university professor enjoying a sabbatical year fell a ready "victim" to our offers. The situation, however, has been gradually changing in recent years and the tendency now is to employ fewer short-term consultants, and to offer more appointments on contracts of up to five years, or even on a permanent basis, though these, regrettably, are still quite few and far between.
In the earlier years of international work there was a great need for advice and assistance on specific matters which could be dealt with on an emergency basis by providing the services of an expert for anything from a few months to a year. Now, countries are realizing that for specialist help to be of maximum value, it must usually be given on a long-term basis. Experts are not only required to do the job, but to train others to do it. It is not of much use going into a country like Ethiopia, producing a hundred thousand doses of rinderpest vaccine and leaving with the applause of a grateful nation ringing in your ears. A hundred thousand doses of any vaccine are used up pretty quickly in a country like Ethiopia, and help of this kind is so ephemeral as not to be worth calling help. The expert has to remain in the country patiently doing his job, keeping the apparatus in working order, and training successive relays of laboratory and field workers in all aspects of the production and use of vaccine.

The trend towards career appointments will undoubtedly continue, and veterinarians between the ages of 30 and 40, with the appropriate experience, will be in growing demand. This is being fostered by the United Nations Special Fund under which we can offer contracts for specific periods, and by the desire of our member countries for appointments of a regional nature which usually merit a permanent contract. The salary and allowances are not unattractive, though they do not by any means compete with the higher levels of professional salaries in the commercial and certain other fields.

I would like to end with a quotation: It is the boy Biondello's speech in "The Taming of the Shrew" where he describes Petruchio's sorry steed:—"...possessed with the glanders and like to mose in the chine; troubled with the lampass, infected with the fashions, full of windgalls, sped with spavins, rayed with the yellows, past cure of the fives, stark spoiled with the staggers, begnawn with the botts, swayed in the back and shoulder-shatten..." and so on.

Except for its equine specificity that is a reasonably accurate description of two-thirds of the world's livestock today—diseased, hungry, parasitic and unproductive. It is a measure of the contribution which the veterinarian can make to world progress and prosperity.
EXPERIMENTALLY INDUCED HOG CHOLERA IN PIGS IMMUNIZED WITH AFRICAN SWINE FEVER VIRUS

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Similarities between hog cholera (HC) and African swine fever (ASF) are recognized as a result of observations reported by Montgomery (1921),1 Steyn (1928),2 Walker (1933),3 Geiger (1937),4 DeKock et al. (1940),5 DeTray and Scott (1957),6 and Maurer et al. (1958).7 These comparisons were made on the basis of clinical signs, and gross and microscopic changes.

Differences between the two diseases based on results of serological studies have been reported by Montgomery (1921),1 Walker (1933),3 Geiger (1937),4 and DeTray and Scott (1955)6 who concluded that anti-HC serum did not confer protection to pigs subsequently inoculated with African swine fever virus (ASFV). Malmquist (1963)9 found that anti-HC serum had neither hemadsorption-inhibiting or precipitating antibodies against ASFV (Hinde).

Simultaneous inoculation of ASF and HC antiserum did not prevent death although the course of ASF was extended according to DeKock et al. (1940).5

Neitz and Burkhart (1952)10 found that neither ROVAC nor crystal violet (HC vaccine) conferred protection against ASFV.

Conceicao (1949)11 concluded that the two viruses were antigenically distinct despite the lack of data on reciprocal immunologic relationships. For example, it was not shown that pigs immunized with ASFV were susceptible to subsequent infection with HC virus (HCV).

Success in modifying the virus of ASF (Malmquist, 196212 and Manso Ribeiro13) has provided an opportunity to further define immunological differences between the viruses of ASF and HC.

This paper describes the immunization of pigs with ASFV and the reaction of immunized animals to inoculation with HCV.

MATERIALS AND METHODS

Viruses

Inocula from three cell culture passage levels, 81, 82, and 109 of modified ASFV (Lisbon, 1960) were used to immunize pigs.*** The 82nd passage was prepared at the Plum Island Animal Disease Laboratory by subinoculation in pig bone marrow cell cultures with inoculum from

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EXPERIMENTALLY INDUCED HOG CHOLERA

passage 81 (Portugal). Virus from passage 109 (Portugal) was injected into pigs without further passage in cell culture. The challenge virus was ASFV (Lisbon, 1960) from the third passage in buffy coat cell cultures (BCCC).*

The HCV (Sn 325) used to infect ASF-immune pigs contained 50,000 pig lethal doses (PLD) per milliliter.**

Antiserum

Anti-HC serum (Sn No. A.L. 2068) was used in virus neutralization tests.**

Anticoagulant

Three and eight-tenths percent sodium citrate was used in collection of blood for use in hemadsorption and virus neutralization tests.

Diluents

Phosphate buffered saline, M-199 (Difco) and plasma collected with anticoagulant from pigs were used in cell cultures, preparation of tissue suspensions, and serological tests.

Cell cultures

Buffy coat and bone marrow cells were used for production and titration of virus and for assay of antibody. The BCCC were prepared in accordance with the method of Hess and DeTray14 and the test procedure described by Malmquist and Hall15 was used. Bone marrow collected from femurs of pigs was suspended in 30 ml. of M-199, pH 7. Tissues were minced with scissors and filtered through two layers of gauze which was rinsed with 10 ml. of M-199. The suspension was placed in 35-ml., round-bottom, centrifuge tubes and centrifuged at 375 x g for ten minutes. The supernatant fluid, including fat, was removed and the cells were resuspended in 40 ml. of M-199. The cell concentration was adjusted to 7 x 10^6/ml. in equal parts of 50 percent M-199 and homologous serum. Cells were dispensed into four ounce prescription bottles.

Pigs

Sixty-pound cross-bred Yorkshire-Tamworth pigs were used for assay of virus infectivity and for inoculation with modified ASFV. Cross-bred Yorkshire-Tamworths, weighing approximately 200 pounds were used as indicators in the virus neutralization tests and bone marrow for cell culture was collected from 100 pound pigs.

Virus neutralization tests

Blood, containing at least 10,000 PLD/ml. of HCV, was collected from pigs at five days postinoculation (DPI). It was mixed with equal parts of

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anti-HC serum and incubated at 37 C for one hour. Pigs were then inoculated intramuscularly (IM) with one ml. of this mixture.

EXPERIMENTAL PROCEDURE

Each of 12 pigs were inoculated (IM) with one ml. of attenuated ASFV (Lisbon, 60). Of the 12 pigs, three were inoculated with virus of the 81st passage, seven with virus of the 82nd, and two pigs were inoculated from passage 109.

Challenge of immunity with ASFV

Five pigs were inoculated IM with virulent ASFV at 35 DPI, and seven pigs were inoculated 20 days after inoculation with modified ASFV. Each pig received one ml. of buffy coat suspension containing approximately 10,000 PLD/ml. of ASFV.

Infection with HCV

Ten immunized pigs that survived inoculation 39 days previously with virulent ASFV were inoculated IM with HCV. Five days later, blood was collected from three pigs for use in a virus neutralization test with anti-HC serum.

Tests for ASFV in the blood of ASF-immune pigs after challenge inoculation with HCV.

Two neutralization trials were made as follows:

Trial 1.—Infective blood collected from ASF-immune pigs five days after challenge inoculation with HCV was diluted 1:100 and incubated at 37 C for one hour with equal parts of undiluted anti-HC serum. Each of four pigs were inoculated IM with one ml. of the infective blood-anti-HC serum mixture; one ml. of the infective blood, without antiserum, was injected into each of two pigs.

Trial 2.—The final dilution of infective blood in this trial was 1:1000. Forty ml. of anti-HC serum was injected subcutaneously (SC) into two pigs two days prior to inoculation with blood-anti-HC serum mixtures. Forty ml. of anti-HC serum was injected into one pig 11 days after inoculation with blood-anti-HC serum mixture. One of the four pigs was inoculated with blood, only.

RESULTS AND CONCLUSIONS

Immunization of pigs with ASFV

Five pigs inoculated with ASFV passages 81 and 109 had no clinical reaction when observed for 35 days before inoculation with the virulent virus. Of seven pigs inoculated with the 82nd passage, six had significant thermal reactions at three to five DPI (Figure 1). Temperatures then returned to normal, and no other clinical signs were observed.
Figure 1. Thermal reaction of six pigs after inoculation with modified ASFV - Lisbon.

Figure 2. Thermal reaction of pigs after inoculation with virulent ASFV - Lisbon 60, Passage 3.
Infection with virulent ASFV

Two of the 12 pigs died after challenge inoculation with approximately 100,000 PLD of ASFV, Lisbon 60. One of the dead pigs had previously been inoculated with modified virus from the 81st passage and the other with virus from the 82nd passage. After challenge inoculation, one pig had a temperature elevation at six DPI and died at 17 DPI; the other had temperature elevation at 17 DPI and died at 34 DPI. Splenic tissue suspensions from both pigs elicited hemadsorption reactions in BCCC.

Five other pigs also responded with elevations in temperature sufficiently above normal to indicate reaction to the challenge virus. (Figure 2). Other than febrile reaction and slight depression for periods of one to three days, no significant changes in the condition of these pigs were noticed.

The two uninoculated control pigs housed with the pigs inoculated with modified ASFV remained unaffected until after the challenge virus was injected into the vaccinated pigs. One of the two pigs died at 22 and the other at 23 days after challenge inoculation of the vaccinated pigs with virulent ASFV.

Infection with virulent HCV

Each of ten ASFV-immunized pigs were given at least 50,000 PLD of HCV 39 days after the ASFV challenge inoculation. All HCV-inoculated pigs died or were killed in extremis within 12 DPI. Lesions resembling those of HC were found in all pigs. The similarity of lesions resulting from infections with HC and ASF viruses as described by Maurer7 is acknowledged; however, the marked hemorrhage frequently involving the visceral lymph nodes of pigs affected with ASF was not found in nodes of the HC-infected pigs. Suspension of spleen from these pigs did not elicit adsorption of red blood cells (RBC) in BCCC.

The above data appeared to offer convincing evidence that pigs immune to ASF are susceptible to experimental infection with HCV as all pigs died following challenge inoculation with HCV.

That death was caused solely by HCV, however, may be considered as less than conclusive, as the diagnosis was based on evaluation of lesions and results of BCCC tests neither of which are recognized as unequivocal differential diagnostic criteria. Pigs recovered from ASF may be viremic and under stress of challenge with HCV; therefore, a viremic state with ASFV might be anticipated. An indirect virus neutralization test to detect ASFV was used to further confirm the singular role of HCV as the cause of death.

Virus neutralization trials

Trial 1.—Of four normal pigs inoculated with HCV-infected blood from ASF pigs and anti-HC serum mixtures, three developed a febrile reaction. Two died despite administration of anti-HC serum (Table I).

Necropsy findings agreed more with those described for HCV than for ASFV. Tissue suspensions did not elicit hemadsorption reaction in BCCC.
TABLE I

<table>
<thead>
<tr>
<th>Test pig number</th>
<th>Inoculum: 1 Blood(^a) and hog cholera antiserum</th>
<th>Inoculum: 2 Hog cholera antiserum at 7 DPI(^b)</th>
<th>Reaction</th>
<th>Febrile</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 ml.</td>
<td>50 ml.</td>
<td>none</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 ml.</td>
<td>50 ml.</td>
<td>106.(^c)</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 ml.</td>
<td>50 ml.</td>
<td>107.(^c)</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 ml.</td>
<td>50 ml.</td>
<td>106.4(^c)</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)50,000 PLD/ml.
\(^b\)Seven days after inoculum 1.
\(^c\)Degrees F at six DPI.

Results of trial 1 of the virus neutralization tests indicated that HCV was not completely neutralized by homologous antiserum. Failure of the serum to neutralize was probably due to HCV being intracellular either in leukocytes, RBC or both; hence, the virus was protected from the action of antibody. The character of these lesions and the fact that the tissue suspensions, as well as the five-day blood, did not elicit hemadsorption reaction in BCCC provided rather conclusive evidence that death was due to HCV and there was no ASFV in the blood.

However, the thermal reactions of two pigs and the death of two others indicated that a less equivocal test would be desirable to confirm that ASFV was not present in the blood after HCV challenge inoculation.

**Trial 2.**—Accordingly, a second virus neutralization trial was conducted in which two normal pigs received 40 ml. of anti-HC serum SC at two days before inoculation with the infective blood-anti-HC serum mixtures. One pig similarly inoculated did not receive anti-HC serum and one pig received infective blood only.

The two pigs, inoculated with virus-serum mixtures, that had received serum before inoculation developed a slight febrile response at seven DPI. However, they returned to normal within two days and remained normal throughout the remainder of the 13-day observation period. The pig that received only infective blood-anti-HC serum mixtures responded with sustained febrile reaction beginning at eight DPI, but the temperature receded following inoculation with anti-HC serum. Thirty ml. of the antiserum was given IM, 10 ml., SC, and ten ml. intravenously (IV).

The pig inoculated only with infective blood developed a febrile response on the fourth day and was killed in extremis at 14 DPI. Tissues taken at necropsy did not elicit hemadsorption reaction in BCCC. The results of this trial confirmed the tentative observations of the first trial. They also justified a definite conclusion that ASFV was not present in the blood of pigs five days after inoculation with HCV.
SUMMARY

1. Modified African swine fever virus (ASFV) stimulated development of homologous antibodies that protected against ASFV challenge inoculation but not against infection with hog cholera virus (HCV). This finding, along with results previously reported, clearly establishes the lack of significant immunological cross reactions between HCV and ASFV. This lack of cross reaction simplifies and strengthens differential diagnosis through cross-protection tests. Sharing of antigens with HCV by ASFV types, other than Lisbon, seems unlikely despite the apparent plurality of antigens among ASFV types.

2. A single dose of modified ASFV protected 10 or 12 pigs against subsequent ASFV inoculation.

3. Thermal reactions in pigs inoculated with the 82nd passage of ASFV were more pronounced than those in pigs inoculated with the 81st passage.

4. Two noninoculated pigs in direct contact with ASFV-inoculated pigs for 22 days did not acquire infection. These pigs did become infected from contact with vaccinated pigs after the latter were inoculated with virulent ASFV.

REFERENCES

A number of significant developments and changes have occurred in the world since the last report of the Committee in respect to those diseases we have rather arbitrarily termed exotic or foreign. We are acutely aware that equine piroplasmosis has become established in some areas in the United States, and that tick vectors are indigenous in particular regions. Evidence is mounting that certain porcine enteroviruses related or identical or classical Teschen, Talfan, or poliomyelitis suum viruses exist in areas of the world where they were not previously thought to exist. Encephalitic symptoms in swine have been observed in the United States for many years, but only recently have some cases been reported as being related to the porcine enteroviruses infections including those related to Teschen disease.

African swine fever, (ASF), introduced into Portugal in 1957, has miraculously confined itself to the Iberian Peninsula. Losses there have been heavy. Recently Spanish workers have reported milder forms of the disease with some survivors. This increases the hazard of spread and the difficulty of eradication since many survivors are carriers and disseminators of ASF virus. Spanish workers have also reported that ticks (Ornithodorus sp,) may act as vectors of ASF virus. This factor would further complicate the control of ASF. The danger of spread of ASF to other areas in Europe and elsewhere is now more imminent than at any time in the history of this disease.

During the past year, foot-and-mouth disease has provided several unusually serious problems. SAT-I-FMD appeared suddenly in the Middle East and seriously threatened Europe. Fortunately, and at least partly as a result of prompt action on the part of the Food and Agriculture Organization (FAO) and other international agencies, the disease has not made inroads into Europe. However, its effect in the Middle East has been most severe...

Eastern Europe and parts of Western Europe were hard hit by Type 'O' FMDV this year, but a great deal of progress was made in most Western European countries in reducing the incidence of FMD. In Africa, Zanzibar had its first FMD outbreak in seven years. Bechuanaland has been plagued by serious outbreaks during the year. In Latin America massive efforts are being made to control FMD. Success in reducing the incidence has at least been temporarily achieved in Argentina and Venezuela through intensive vaccination. In many areas of the world, great
interest is being shown in the potential value of modified live virus vaccines as a tool in FMD control. The field trials involving utilization of hundreds of thousands of doses of modified vaccines in Africa, the Middle East, and Latin America may indicate whether or not more efficacious methods of controlling FMD are near at hand.

Notable advances have been made in research and investigations on scrapie and other diseases of probable viral origin with long incubation periods.

The combined effect of a massive vaccination program and the extensive epizootic of African horsesickness in the Middle East during 1960-61 has resulted in a decline in the incidence of this disease in that area. In Africa, the isolation of two "new" strains of AHS virus, one from Africa and one from the Middle East, forewarn the possibility of further problems in immunization against this disease.

Trypanosomiasis appears to be expanding its distribution in Africa although major reclaimed areas have not become reinfected.

Rinderpest is on the decline in most areas of the world as a result of markedly increased immunization programs.

Some evidence now exists that bluetongue has extended its range to South America and there is some indication that the spread of this insidious disease may be occurring in Asian areas also.

This Committee has undertaken an extensive task in revising the handbook *Foreign Animal Diseases* and a number of sections have been redrafted and are being distributed for commend and criticism. It is anticipated that most drafts of sections to be included in the revision will be reviewed by the first of the year and that the entire material will be readied for publication during 1964.

Efforts have been made to obtain financial support for publication, but it appears likely that the original cost will have to be borne by the Association and recouped through sales. In addition to sections and appendices dealing with animal disease control requirements and procedural recommendations, the following diseases will be treated:

- African Horsesickness
- African Swine Fever
- Anthropod-borne Protozoan Diseases
- Anthropod-borne Virus Diseases
- Borna Disease
- Bovine Infectious Petechial Fever
- Contagious Agalactia
- Contagious Bovine Pleuropneumonia
- East Coast Fever
- Enzootic Abortion
- Ephemeral Fever
- Foot-and-Mouth Disease
- Fowl Plague
- Heartwater
- Hemorrhagic Septicemia
- Infectious or Epizootic Infertility of Cattle
- Louping Ill
- Lumpy Skin Disease
- Melioidosis
- Nairobi Sheep Disease
- Rift Valley Fever
- Rinderpest
- Sheep Pox
- Sweating Sickness
- Teschen Disease
- Trypanosomiasis
- Venezuelan Equine Encephalomyelitis
The practicing or Civil Service Veterinarian is familiar with livestock diseases commonly found in the United States and Canada. He must however, familiarize himself with the foreign animal diseases which do not occur in our countries, and have readily available reference to information on these diseases if he is to properly discharge his professional responsibilities. Diseases considered to merit special consideration in this connection are described in the following chapters.

Reporting

A prompt reporting system is the first and most important step in the control and eradication of exotic diseases of livestock and poultry. The early recognition of a potential outbreak followed by an immediate report could well be the difference between a short and economical eradication effort or a long and costly control program.

The livestock owner and the practicing veterinarian are usually the first to see evidence of infection. They have the first responsibility to report their observations.

The following chain of communications to initiate rapid action on these reports has been set up by USDA and State regulatory officials.

1. The farmer who observes an unusual disease in his livestock should report it immediately—either to his local veterinarian or directly to the Chief State Livestock Sanitary Official.

2. The state official and the USDA Veterinarian—in-charge conducts or directs the conduction of a preliminary investigation of the case.
3. If findings warrant it, they arrange for the assistance of a USDA diagnostician specially trained in the diagnosis of foreign diseases of livestock and poultry.

4. The diagnostician immediately reports his observations to State and Federal officials and a decision is made on a course of action. The course of action may range from a quarantine for further observation to the submission, by courier, of tissue and/or serum samples to the Plum Island Animal Disease Laboratory for diagnostic tests.

5. If the disease is confirmed to be dangerous animal disease foreign to the U.S., State Livestock Sanitary authorities establish quarantines and join with Federal regulatory officials in activating emergency control and eradication procedures.

All communication between persons responsible for the reporting and diagnosis of the suspected disease condition is by telephone to expedite the immediate action needed to obtain a confirmed diagnosis as rapidly as possible. Figure 1 illustrates the channels for reporting and securing diagnosis of foreign animal diseases.
FOREIGN ANIMAL DISEASES

PREFACE

Since the publication of Foreign Animal Diseases by the United States Livestock Sanitary Association in 1954, a great many changes have occurred. The distribution and significance of some have increased and control measures for some have improved. New knowledge regarding many of the diseases has been developed and a few, during the interval, have become established in the United States. This new report is intended to bring material in the 1954 publication up-to-date and add significant new information.

It is essential that practicing veterinarians dealing ordinarily with area problems have at their disposal a manual or guide dealing with diseases in other areas of the world that might, quite suddenly, affect or threaten the health and welfare of the local livestock.

Changes in regulations regarding the protection measures, reporting systems and emergency actions have also occurred in the years since the Association published the first version of this report, and the sections dealing with these matters have also been revised.

Much of the information contained in this report has been collected only recently and some is based on personal experiences and observations that have been provided by direct communication. Therefore, such information is not readily available anywhere else in the country at the present time.

It is hoped that this report will provide veterinarians concerned with the protection of our domestic livestock, concise knowledge of some of the most important exotic diseases and enable them to react promptly in detection and control efforts in the event of outbreaks.

For those with more than cursory interest, wishing to pursue studies of these diseases in more detail, an effort has been made to include a reasonably adequate and current guide to the literature on the subject.

INTRODUCTION

The history of mankind from ancient times contains references to animal diseases and epizootics. In early cultures this evidence was depicted pictorially and, as civilization advanced, organized societies, including those of China, Egypt, Phoenicia, Greece and Rome, placed in literary descriptive terms the signs and consequences of animal ailments.

Wild tribesmen, Tartar hordes, Roman legions, crusading knights, Napoleon's armies, as well as modern-day armies, have contributed to the spread of animal diseases. Viking ships moving out of Scandinavia to settle Iceland and Greenland; conquistadors and pirates, pilgrims and colonizers, undoubtedly brought new diseases and vectors to new areas, and it is only through fortuitous circumstances or naturally unfavorably ecological conditions that even more were not introduced in such places.
These early travelers knew little of disease agents, were unaware of modern concepts of sanitation, and consequently brought unseen stowaways—disease agents or vectors—with their animals or seed stocks.

Today, with movement of people, plants and animals vastly magnified, the potential for disease dissemination is multiplied many fold, and it is only constant vigilance, surveillance, and restrictive regulation that prevents the free passage of agents regularly between countries or regions.

During the modern development of medical sciences, it has become apparent that migratory animals and birds, possibly wind and sea currents, inapparent carriers and unusual hosts play a part in the emergence of diseases in new areas. Some of these disease agents may be of low virulence or subtly occult in a nonsusceptible host, and on finding susceptible species in a favorable environment revert to high virulence and invasiveness, thus initiating new enzootics or epizootics.

With these constant changes and an ever increasing food requirement for a worldwide expanding population—approximately 50,000,000 per year—it is estimated that the requirement for animal products will be higher by nearly 200 percent at the turn of the century.

Disease control is a vital and essential factor in increasing supplies of food of animal origin, and with increasing demand the attention to improved methods, alert recognition, and prompt action becomes more and more essential. Diseases least familiar, often described as exotic, cannot only be the most devastating but also the most insidious, gaining a firm foothold before practical methods of control or eradication can be initiated.

The veterinary profession as a whole has the inescapable responsibility for preventing disastrous disease outbreaks. Government regulatory agencies have the responsibility for organizing and directing emergency disease control programs, but veterinarians in practice and other phases of veterinary science form an essential part of the total force necessary to carry out emergency measures as well as the nation's normal animal health requirements.

To accomplish these charges effectively, it is essential that veterinarians know not only something of the diseases that constantly threaten our livestock population, but that they also recognize the nature and scope of preventive measures necessary to control or eradicate outbreaks.

Because of increased and expanded international trade and travel, the existence of a disease in our part of the world poses a threat to another. Foreign animal diseases can no longer be regarded as exotic curiosities of purely academic interest. If we are to cope with the apparent and inevitable extension of diseases within our own boundaries and participate in the knowledge of a host of unfamiliar diseases and our present efforts of containment or control must be vastly improved.
FOREIGN ANIMAL DISEASES

CONTAGIOUS BOVINE PLEUROPNEUMONIA

Identification of Disease

a. Definition. Contagious bovine pleuropneumonia is a highly infectious, acute, sub-acute or chronic septicemic disease of cattle caused by an extremely small bacterium,

b. Etiology. *Mycoplasma mycoides* (*Asterococcus mycoides*, *Borrelomyces peripneumoniae*), once classed among a group of related organisms as a virus or as "virus-like," is now recognized as a pleomorphic, very small bacterium which in some of its forms may be filtrable. The organism is sensitive to drying and heating as well as other environmental influences. It is readily susceptible to disinfectants and does not survive outside the animal body in nature for more than a few hours.

The causative organisms of contagious pleuropneumonia in cattle and goats are similar culturally and antigenically but do not spread between the species.

c. History. Contagious bovine pleuropneumonia is enzootic in many large areas of Australia, Africa and Asia. The disease still exists in Spain but other European countries, including the U.S.S.R., claim that the disease has been eliminated.

Contagious bovine pleuropneumonia was introduced into the United States in 1843, and possibly on two subsequent occasions. Its rapid spread westward, in 1886, led to the establishment of the Bureau of Animal Industry of the U.S. Department of Agriculture in 1887 and the first all out disease eradication program. The last known cases in the Western Hemisphere occurred in the United States in 1892.

South Africa eradicated the disease in 1916, after over half a century of serious losses resulting from the introduction of an infected bull from Holland.

In Australia, Tasmania, New South Wales, Victoria are free, and Western Australia below latitude 20° is free except for occasional introductions of carrier animals from enzootic regions. These periodic disease introductions are quickly eliminated.

Communist China and India both report the disease, but do not consider it a serious threat. Japan reported the disease eradicated in 1941.

Symptoms

a. Clinical features. Clinically, disease develops with a sudden rise in temperature (105°F.), anorexia, cessation of rumination and severe depression. At first coughing occurs only after exercise. Chest pain, with symptoms of arched back, distended elbows and extended head and neck, is obvious. Respiratory signs of grunting expiration, shallow rapid breathing and fluid sounds appear. As the disease progresses auscultation reveals gurgling rales, pleuritic friction, and areas of dullness in the lung are evident on percussion. Edematous swelling of the throat and dewlap may occur.
b. **Incubation period.** In natural conditions the disease develops in as little as 10-14 days, but the average mean period in contact animals under observation has been 120 days. The disease ordinarily spreads slowly, but rapid communication has been recorded on numerous occasions.

**Pathological Changes**

a. **Post-mortem lesions.** Gross pathological changes are striking in contagious bovine pleuropneumonia. The disease begins with catarrhal bronchiolitis and a serofibrinous lobar and interstitial pneumonia. In active cases a thickening and inflammation of the pleura with heavy fibrin deposits occur. Gross effusion of clear or pinkish serous fluid is generally apparent. One or both lungs show marked interlobular edema, inflammation of the mucous membranes of the bronchioles and often, in terminal stages, marked areas of pleural adhesions. As the interlobular septa become distended stages of hepatization from red to gray may occur, producing the classical "marbled" lung of the disease. In more chronic forms, or in apparently recovered animals, necrotic areas may be walled off by connective tissue capsules forming characteristic sequestra which may persist for a long time. These may eventually be absorbed or become liquefied abscesses.

b. **Histological changes.** An outstanding histological change in the disease is the separation of the lung lobules into distinct compartments by the heavily thickened interlobular septa. Lobules may contain areas of intact alveoli, but in others consolidation is complete. Intense infiltration of lymphocytes and plasma cells is seen around blood vessels and bronchi. Leukocytes also concentrate with the interlobular septa.

**Diagnosis**

a. **In the field.** During life it may be difficult to distinguish cases of contagious bovine pleuropneumonia from other forms of pneumonia with pleuritic involvement. Several investigators have called attention to possible similar clinical or pathological changes to those occurring in acute hemorrhagic septicemia caused by *Pasteurella multocida* Type I Roberts and in certain types of mechanical pneumonia. These more or less parallel changes are generally confined to early stages of the disease and history along with a number of serological reactions serve to differentiate CBPP from other diseases. Observation of the more or less typical mosaic or marbled appearance of affected lobules and the presence of a quantity of straw-colored fluid in the thorax should lead to a suspicion of contagious bovine pleuropneumonia.

b. **In the laboratory.** Antibodies appear in the blood early in the course of the disease. Various reactions, including complement fixation, agglutination and precipitation tests, are used in diagnosing the disease. The complement fixation test is generally considered accurate and produces few false positive reactions if properly conducted. Variations of these tests are also used. Agar-gel-diffusion, indirect hemagglutination, and allergic tests are being studied.
The tube precipitin test used by German and Japanese workers several years ago is now considered useful because it gives satisfactory results on tissue stored in formalin over long periods and on relatively putrid material. The agar-gel-diffusion test can also be used for solid tissue and decomposed material.

Experts agree that development of a satisfactory allergic test would be a great aid in field diagnosis and control.

Pleomorphic forms of the organism can be observed in microscopic observations of freshly collected fluids. A little exploratory work has been done in Australia on the fluorescent antibody techniques as a means of identifying the organism in tissues.

**Prognosis**

In acute cases death usually occurs in two to three weeks after appearance of the first symptoms. Epizootics may produce mortality rates varying from 10 to more than 70 percent, but generally 50 percent of animals in a herd may show some clinical symptoms, and most of these will make an apparent recovery.

**Epizootiology**

*a. Geographic distribution.* Contagious bovine pleuropneumonia occurs in many parts of Africa, particularly in East Africa, Central Africa, Ethiopia and the Sudan, Central Asia including India, the U.S.S.R., Spain, and an area of Australia mainly confined to the northern fifth of the continent and a part of southwest Queensland.

*b. Transmission.* The disease is spread by inhalation of finely dispersed bronchial secretions resulting from coughing. Transmission by ingestion and other routes in nature is not considered possible. Foci of infection in new outbreaks may be "recovered carrier" animals where pulmonary sequestra in apparently healthy animals break down under conditions of stress, such as exhaustion, starvation or intercurrent disease. Such carrier stock may harbor the organism for as long as three years without apparent clinical signs.

*c. Communicability.* Cattle of all ages are affected, but the degree of susceptibility among strains or types may vary considerably. Buffalo, yaks, bison, antelope and reindeer are considered resistant but natural infection has been recorded in these species. Sheep and goats never contract natural infection and may be artificially infected only by subcutaneous exposure and the organism acts in producing a septicemic reaction.

**Control**

*a. Preventive measures.* Contagious bovine pleuropneumonia is a particularly insidious disease in that an extended carrier state exists in a large share of apparently recovered animals. Such a situation calls for special regulations and investigative procedures in particular areas, depending upon the character of animal husbandry and marketing systems.
Several tests have been developed to identify affected animals. These tests are not entirely satisfactory for all circumstances. An International Joint FAO/OIE/CCTA Expert Panel on the disease has recommended further research to improve diagnostic techniques, particularly those considered most useful as aids in confirming field diagnosis.

In event of introduction of the disease into new areas, the most effective method of dealing with the situation is prompt isolation of exposed breaks should be quarantined and frequent tests applied over at least a year to determine their status.

b. Treatment. In some areas of the world where the disease is enzootic, chemotherapy is used as a means of salvaging valuable animals and to combat adverse vaccinal reaction. Sulfonamides and antibiotics, such as streptomycin, oxytetracycline and choramphenicol have proved useful.

c. Sanitation and disinfection. In enzootic areas newly affected and carrier animals should be quickly isolated to protect healthy cattle from infection. Special disinfection is unnecessary since the organism is quickly destroyed in nature and infection by aerosol droplet infection is considered the sole possible mode of infection. There is apparently no risk of transmission to new areas through importation of meat or meat products.

d. Immunization. Immunization is a common practice in countries where the disease is enzootic. However, the variable susceptibility of different strains of cattle, the lack of immune response in significant numbers of vaccinates, and the frequent serious reactions to vaccination leave doubt as to the advisability of using any of the numerous inactivated, living culture or avianized vaccines as the sole method of eliminating the disease.

The inactivated vaccine used in large doses induces a degree of immunity but overall results are inconclusive. Its use in cattle highly susceptible to other vaccines may be advisable until more effective immunizing agents materialize.

Living culture vaccines produced from various apparently fixed M. mycoides strains may lose immunizing properties under continued transfer in media. The occasional reversion to greater virulence has also been noted. The most serious disadvantage is limited keeping quality. Living culture vaccine adjuvants have not been adequately studied. They have the advantage of reducing the number of organisms required to produce immunity.

Avianized vaccines appear to offer good possibilities for immunization. However, serial passage of different M. mycoides isolates in chick embryos results in inconstant levels of attenuation at various levels. Some isolates apparently become avirulent; others maintain virulence after repeated passage. Various routes of inoculation have been studied, including tail tip, subcutaneous and intramuscular. Recently a route of vaccine inoculation for any of the pleuropneumonia vaccines, except those formalized, in the mid line of the muzzle at the mid point between
inferior-internal angles of the nares has been recommended in Central Africa. Vaccination by this route has not produced serious vaccinal re-
action nor have lung lesions in vaccinates been noted. The efficiency of this type immunization has not yet been fully assessed.

There is little doubt that immunization will continue to play an im-
portant part in the control of this disease in enzootic areas. Nevertheless a great deal more work is necessary to perfect vaccines that will satisfy the variable requirements for diversely susceptible types of cattle exposed to different strains of the organism.

Public Health Aspects

There is no evidence that man is susceptible to this disease.

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EXPERIMENTAL *LEPTOSPIRA POMONA* INFECTION IN THE CHINCHILLA (CHINCHILLA LANIGER)∗

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During the studies undertaken to determine the basic mechanism of hemoglobinuria observed in cattle infected with *Leptospira pomona* it was observed that the chinchilla became more susceptible to challenge after vaccination with a specific bacterin (Bacterin A) than did the controls.

It has been postulated that bacterial cell substances may act as a sensitizing as well as an immunizing agent. Morter postulated that the hemolytic effect in leptospirosis in sheep is due to interaction of a leptospiral intracellular component, antibody, and red blood cells. This type of hypersensitivity to leptospiral organisms has not been previously reported.

**MATERIALS AND METHODS**

Three trials were conducted using the chinchilla as the test animal. This species was selected as it has been reported to be more susceptible to *L. pomona* when compared with the young guinea pig and hamster.¹⁻³

Nineteen chinchillas were selected at random varying in age from six months to three years. Ten of these served as vaccinees and were injected intramuscularly with 0.5 milliliters of bacterin A. This amount represented one fourth of the recommended dose for adult bovines and was considered adequate to produce resistance to challenge in this laboratory animal.

The vaccinees and controls were challenged 18 days later with a field strain of *L. pomona* isolated from a six-month-old calf by the authors. The strain was typed at the Army Medical Center, Washington, D.C. Modified Stuart's media was used to maintain the leptospira and virulence maintained by routine passage through the chinchilla. Virulence of the strain at the time of challenge was such that a thermal response was elicited and clinical signs were consistently produced as early as the third day after inoculation with death resulting by the fourth to sixth day. The challenging dose contained approximately 250 million organisms in 0.25 milliliter and given intraperitoneally.

In the second trial 32 chinchillas were selected as above. Twenty-four served as vaccinees and eight as controls. Twelve of the vaccinees received one dose of bacterin A intramuscularly while the remaining 12 received two doses intramuscularly, fifteen days apart. Both groups of vaccinees and controls were challenged 70 days later. The challenge

*Contribution No. 210, Station Annual No. 68, Kansas State University, Agricultural Experiment Station, Department of Veterinary Science.

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LEPTOSPIRA POMONA INFECTION IN THE CHINCHILLA

consisted of an LD-50 containing approximately 600 organisms in 0.25 milliliters of medium and injected intraperitoneally.

Thirteen chinchillas were selected as above for the third trial. Eight served as vaccinees and were given 0.5 milliliters of bacterin B intramuscularly and five were selected as controls. All animals were challenged 70 days later with the LD-50 as in trial II.

Blood samples were collected before vaccination and prior to challenge for serological studies. Blood cultures were collected from the heart on initial thermal response and inoculated into modified Stuart's medium. Tubes were incubated at 29°C and examined on the fifth day for leptospira by darkfield and weekly thereafter for a period of 33 days. Temperatures were taken four days prior to vaccination and daily thereafter until the trial was terminated. Only one or two animals were kept in a single cage and under normal laboratory conditions.

Tissues were collected and fixed in 10 percent formalin and stained by the Warthin-Starry technique.

RESULTS

Serological titration (agglutination-lysis) of sera from animals in Trials I and II did not indicate an increase in serum titres following vaccination with bacterin A. Majority of the samples showed a negative titer both before and after vaccination. Only 10 of the vaccines gave a slight reaction in dilution of 1-10. Serum titres in Trial III with bacterin B showed a greater response, highest titer being 1-6250.

The vaccinees 10/10 and all controls 9/9 in Trial I died following challenge. The average time of death for the 10 vaccinees was 4.5 days and the controls 5.1 days. It was concluded from these results that the challenging dose was too great and was therefore reduced to an LD-50 in Trials II and III. In Trial II, 12/12 vaccinees receiving a single dose of bacterin A died and 10/12 vaccinees receiving two doses of bacterin A. Only 3/8 controls died following challenge. In the third trial all vaccinees 0/8 survived challenge and 3/5 controls died.

The temperature response in the vaccinees receiving one dose of bacterin A was consistently higher than controls (average temperature 102.5 and 101.6). Positive blood cultures were obtained from 93 percent of all animals in trials I and II. Positive blood cultures were not obtained from the vaccinees in Trial III, but all the controls gave positive blood cultures.

Clinical signs observed were depression, lassitude, anorexia, oliguria, anuria, and roughened hair coat. The gross lesions consisted of jaundice, extensive hemorrhage on the serosal surfaces, kidney, lungs, and stomach. Free blood was present in the stomach of many of the animals that died. Hemorrhage in the psoas muscle was also a frequent finding. These findings were more severe and extensive in the vaccinees than in the controls.

Microscopically the kidney in acute cases revealed a necrotizing exudative glomerulonephritis with fibrinoid degeneration of the tufts. The capillary walls of the glomeruli were thickened and hyalinized areas were
often prominent. Most of the capillary loops of such glomeruli were devoid of blood. These findings are associated with tissue allergy but may also be found in conditions where hypersensitivity is not suspected. Severe degenerative changes occurred in the tubules following the early stages of infection. Leptospiral organisms were observed in liver and kidney sections.

DISCUSSION

It is apparent from the exaggerated clinical signs and microscopic findings in the nonimmune vaccinees that the chinchilla may become hypersensitive to leptospiral organisms or their products of disintegration in vivo or vitro. These observations may help explain the occasional severe outbreak of leptospirosis in cattle following field vaccination.5

This report is not complete in many respects, but it helps to raise the following question: Could this state of hypersensitivity, when it occurs, result from natural exposure to L. pomona as well as be induced by biological products prepared from those organisms?

The results of field observations and laboratory surveys indicate that the majority of leptospiral infections in farm animals occur in a subclinical form and are never observed by the veterinarians or the owners. Surveys in this laboratory indicate that the incidence of this disease varies from year to year; four to 48 percent of blood samples from cattle submitted for brucellosis test were found positive to leptospirosis by agglutination-lysis test.

Hypersensitivity reactions may be a key to the varied signs and lesions observed in this disease.

REFERENCES

SEROLOGICAL RELATIONSHIP BETWEEN BRUCELLA ABORTUS AND LEPTOSPIRA POMONA

W. L. Downey, V.M.D. and R. L. Morter, D.V.M., Ph.D.

Field observations suggest that immunization for leptospirosis or infection with *Leptospira pomona* can result in an increase in the brucellosis titer of cattle. A serological relationship between *Brucella abortus* and *L. pomona* was not described in the literature reviewed. Several serological surveys of wildlife, notably deer, have been made to determine the incidence of *Brucella* and *Leptospira* antibodies, but the reports reviewed have not mentioned the occurrence in the same animal of antibodies to both of the etiological agents.¹²³

Homologous antigen-antibody reactions or true cross reactions have been shown to exist between *Brucella* and several other genera. The relationship between *Br. abortus* and *Pasteurella multocida* has been reported by Mallman, Berman, King and others. Kiggins et al. (1955) studied the relationship between *Br. abortus* and *Vibrio fetus*. Morse and co-workers investigated cross agglutination between *Brucella* species and *Vibrio fetus*, *Vibrio comma*, *Salmonella pullorum* and *Pasteurella tularensis*. A relationship also has been demonstrated between *Brucella suis* and *Proteus* (OX 19).

This research was undertaken to determine if experimental exposure of cattle to *L. pomona* would increase the *Brucella* titer and, if so, to evaluate possible serological relationships between the two organisms.

MATERIALS AND METHODS

Twelve calves of either dairy (11 calves) or beef breeds (one calf) were purchased at three days of age from herds observed to be free of detectable brucellosis and leptospirosis. These calves had received colostrum from their dams. They were maintained in disease free surroundings and castrated at three months of age. At the initiation of the experiment, the calves were five to eight months old.

The 12 steers were bled three times, at three day intervals, to evaluate the *Brucella* and *Leptospira* agglutinin titers and randomly assigned to three groups of four calves each. Two groups of calves were exposed to *L. pomona*; the group receiving *L. pomona* (Ohio) was designated as group LO and the group receiving *L. pomona* (Wickard) as LW. The third group was injected with *Br. abortus* (strain 19) vaccine and identified as

Submitted as Journal Paper 2235, Purdue University Agricultural Experiment Station, Lafayette, Indiana.
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Supported in part by United States Public Health Service Grant AI 04029-03.
group B. The calves in group B later received commercially prepared *L. pomona* bacterins and were subsequently identified as BL.

Serum titers for *L. pomona* were determined by the microscopic agglutination test and for *Br. abortus* by the tube agglutination test. In the microscopic agglutination test, a separate pipette was employed for each of the tenfold dilutions. Five to seven-day-old cultures of *L. pomona* (Johnson) were used as antigens. After two hours incubation at 37.5°C., the tests were interpreted by dark field illumination at a magnification of 125×. The standard *Brucella* tube test was modified by using two-fold dilutions of an initial 1:6.25 serum dilution.

Group LO was exposed by subcutaneous injection of two ml. of heparinized blood obtained by cardiac puncture of leptospiremic guinea pigs. The calves in group LW were exposed by subcutaneous inoculation with one and one-half ml. of heparinized, infected hamster blood. The *Br. abortus* (strain 19) vaccine was commercially prepared. Five ml. were injected subcutaneously into each of the calves comprising group B. Six months later when their brucellosis vaccination serum titers had stabilized, complete agglutination at 1:100 and below, the group B calves were injected twice with Leptogen (Pitman-Moore Company) and a third time with Anti-lepto (Corn States Laboratories, Inc.) and thereafter designated as group BL.

Infection was confirmed in group LO by the inoculation of guinea pigs with urine from the calves. The development of an agglutinin titer for *L. pomona* by the guinea pigs was considered indicative of leptospira in the urine of the calves. Infection of group LW was confirmed culturally for each of the four calves. Hemocultures were prepared in Chang's or Fletcher's fluid media during the acute leptospiremic phase (post-exposure days nine and 10) of the disease, incubated at 28°C., and examined by dark field microscopy at weekly intervals for four weeks.

Blood was collected aseptically from all 12 animals and from at least two collections serum, in quantities adequate for absorption studies, was harvested and stored at -20°C. The *Leptospira* and *Brucella* agglutinin titers were determined by the microscopic agglutination and *Brucella* tube agglutination tests respectively.

The antigens employed for cross absorptions were *Br. abortus* (strain 1119-3) and *L. pomona* (Johnson). *Br. abortus* (1119-3) is the tube agglutination test antigen distributed by the United States Department of Agriculture, Agricultural Research Service, Animal Disease Eradication Division. This antigen is distributed in concentrated form containing four and one-half percent cells by volume. Packed *Br. abortus* cells, removed from the antigen concentrate by centrifuging at an R.C.F. = 12,100 x g., were used in the absorption procedure.

*L. pomona* (Johnson) cells for use as absorbing antigen were cultured at 28°C. in Chang's medium dispensed in Roux flasks in 400 ml. amounts. When maximum growth was reached, 10 percent buffered formalin was added to the medium resulting in a final concentration of one percent formalin; the leptospirae were left in contact with the formalin 24 hours. The cells, recovered from the medium by centrifugation at an
R.C.F. = 30,900 x g., were suspended in phosphate buffer equivalent to one percent of the original volume of medium and stored at -20°C.

Each serum was absorbed with both antigens and tested for the presence of homologous and heterologous antibodies by the microscopic agglutination and tube agglutination tests. All sera were absorbed at 37°C, with continuous agitation for four hours followed by standing 12 hours at 4°C.

Brucella antibodies were absorbed from the various sera by mixing two ml. of antiserum, two ml. 0.85 percent saline with 0.5 percent phenol and the packed cells from two ml. of concentrated Brucella antigen. Following one absorption, Brucella agglutinins were not detectable in a serum dilution of 1:6.25.

Leptospira antibodies were absorbed by mixing two ml. of antiserum, two ml. of Stuart's medium base16 and the packed cells from four ml. of L. pomona (Johnson) suspension. It was necessary to repeat the absorption procedure four times to render the antisera of groups LO and LW negative to the microscopic agglutination test, whereas, one absorption was usually adequate for the sera of group BL.

All sera showing an agglutinin titer for Brucella were subjected to the acidified plate antigen test17 at a pH of 3.75 and to the 65°C heat inactivation test.18

RESULTS

The pre-treatment sera of all 12 animals were negative to the Leptospira and Brucella agglutination tests. All eight animals in groups LO and LW developed agglutinins against L. pomona and six of the eight animals developed Brucella agglutinins. The Leptospira agglutinin titers ranged from 10² to 10⁴. The Brucella titers of these same sera ranged from no agglutination at a 1:6.25 dilution to incomplete agglutination at a 1:50 dilution (Figure 1).

Absorption of these sera with L. pomona (Johnson) and with Br. abortus reduced the homologous titer to a negative reading. The Brucella titers were not affected by absorption with the L. pomona antigen nor were the Leptospira titers affected by absorption with the Brucella antigen (Figure 2).

Since a slight drop in Leptospira titer might have occurred that was too small to be detected by a ten-fold dilution scheme, several sera were tested by the microscopic agglutination test using two-fold dilutions beginning with a dilution of 10⁻³. After absorption with Br. abortus antigen, the test was repeated and the titers for leptospiral agglutinins were found to be the same as the pre-absorption titers.

The maximum end points of Brucella agglutinin titers from the calves of group B prior to absorption ranged from complete agglutination at a 1:400 dilution to complete agglutination at a 1:800 dilution (Figure 3). At no time did these calves develop a detectable serum agglutinin titer for L. pomona. Absorption of these sera with L. pomona (Johnson), repeated four times, failed to affect the Brucella titer (Figure 4).
### TITER OF UNABSORBED SERA FROM *L. pomona* INFECTION CALVES

<table>
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<tr>
<th>GROUP</th>
<th>CALF NUMBER</th>
<th>DAY POST INOC</th>
<th>END POINT TITER</th>
<th>LEPTOSPIRA*</th>
<th>BRUCELLA</th>
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<td></td>
<td>4</td>
<td>84</td>
<td>10^-4</td>
<td>INCOMPLETE</td>
<td>1 : 25</td>
</tr>
<tr>
<td>LN</td>
<td>1</td>
<td>105</td>
<td>10^-2</td>
<td>COMPLETE</td>
<td>1 : 6.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>105</td>
<td>10^-2</td>
<td>INCOMPLETE</td>
<td>1 : 25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54</td>
<td>10^-3</td>
<td>INCOMPLETE</td>
<td>1 : 25</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>105</td>
<td>10^-3</td>
<td>INCOMPLETE</td>
<td>1 : 6.25</td>
</tr>
</tbody>
</table>

* HIGHEST DILUTION SLOWSHEWING AT LEAST 50% AGGLUTINATION-LYSIS

Figure 1

### CROSS ABSORPTION OF *L. pomona* POSITIVE SERA

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CALF NUMBER</th>
<th>DAYS POST INOC</th>
<th>END POINT TITER-AFTER ABSORPTION WITH LEPTOSPIRA</th>
<th>LEPTOSPIRA REACTION</th>
<th>DILUTION</th>
<th>BRUCELLA REACTION</th>
<th>DILUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LEPTOSPIRA</td>
<td>BRUCELLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>REACTION</td>
<td>DILUTION</td>
<td>REACTION</td>
<td>DILUTION</td>
<td></td>
</tr>
<tr>
<td>LO</td>
<td>1</td>
<td>114</td>
<td>NEGATIVE</td>
<td>1 : 6.25</td>
<td>10^-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>114</td>
<td>INCOMPLETE</td>
<td>1 : 50</td>
<td>10^-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>NEGATIVE</td>
<td>1 : 6.25</td>
<td>10^-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>84</td>
<td>INCOMPLETE</td>
<td>1 : 25</td>
<td>10^-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM</td>
<td>1</td>
<td>105</td>
<td>NEGATIVE</td>
<td>1 : 12.5</td>
<td>10^-2</td>
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<tr>
<td></td>
<td>2</td>
<td>105</td>
<td>COMPLETE</td>
<td>1 : 6.25</td>
<td>10^-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54</td>
<td>INCOMPLETE</td>
<td>1 : 25</td>
<td>10^-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>105</td>
<td>INCOMPLETE</td>
<td>1 : 6.25</td>
<td>10^-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2
RELATIONSHIP BETWEEN *BR. ABORTUS* AND *L. POMONA*

**TITER OF UNABSORBED SERA FROM BRUCELLA VACCINATED CALVES**

<table>
<thead>
<tr>
<th>CALF NO.</th>
<th>DAYS POST INOC.</th>
<th>END POINT TITER</th>
<th>LEPTOSPIRA</th>
<th>BRUCELLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>REACTION</td>
<td>DILUTION</td>
<td>REACTION</td>
</tr>
<tr>
<td>B 1</td>
<td>92</td>
<td>NEGATIVE</td>
<td>10⁻¹</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>B 2</td>
<td>92</td>
<td>NEGATIVE</td>
<td>10⁻¹</td>
<td>INCOMPLETE</td>
</tr>
<tr>
<td>B 3</td>
<td>92</td>
<td>NEGATIVE</td>
<td>10⁻¹</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>B 4</td>
<td>92</td>
<td>NEGATIVE</td>
<td>10⁻¹</td>
<td>POSITIVE</td>
</tr>
</tbody>
</table>

Figure 3

**CROSS ABSORPTION OF *BR. ABORTUS* POSITIVE SERA**

<table>
<thead>
<tr>
<th>CALF NO.</th>
<th>DAYS POST INOC.</th>
<th>END POINT TITER-AFTER ABSORPTION WITH LEPTOSPIRA</th>
<th>END POINT TITER-AFTER ABSORPTION WITH BRUCELLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEPTOSPIRA</td>
<td>BRUCELLA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REACTION</td>
<td>DILUTION</td>
</tr>
<tr>
<td>B-1</td>
<td>92</td>
<td>COMPLETE</td>
<td>1 : 800</td>
</tr>
<tr>
<td>B-2</td>
<td>92</td>
<td>COMPLETE</td>
<td>1 : 800</td>
</tr>
<tr>
<td>B-3</td>
<td>92</td>
<td>COMPLETE</td>
<td>1 : 400</td>
</tr>
<tr>
<td>B-4</td>
<td>92</td>
<td>COMPLETE</td>
<td>1 : 400</td>
</tr>
</tbody>
</table>

Figure 4

Group BL was bled at three day intervals for 21 days following each *L. pomona* vaccination and *Brucella* serum titers were determined. No rise in *Brucella* titer was detected.

The end point titers of antisera collected from group BL 40 days after the first vaccination and 13 days after the second vaccination with *L. pomona* bacterin are shown in Figure 5. Figure 6 shows the results of absorption of these sera with both antigens. Absorption with *L. pomona* and *Br. abortus* removed detectable homologous agglutinins but the heterologous agglutinin titers were not affected.

The acidified plate antigen and heat inactivation tests performed on the unabsorbed sera of groups LO and LW were negative. These tests were positive when performed on the unabsorbed sera of groups B and BL.
TITER OF UNABSORBED SERA FROM CALVES VACCINATED WITH Br. abortus (strain 19) AND L.pomona BACTERIN

<table>
<thead>
<tr>
<th>CALF NO.</th>
<th>DAYS* POST INOC.</th>
<th>END POINT TITER</th>
<th>LEPTOSPIRA**</th>
<th>BRUCELLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEUKOSPIRA REACTION</td>
<td>DILUTION</td>
<td>LEUKOSPIRA REACTION</td>
</tr>
<tr>
<td>BL 1</td>
<td>40</td>
<td>POSITIVE</td>
<td>10^-2</td>
<td>INCOMPLETE</td>
</tr>
<tr>
<td>BL 2</td>
<td>40</td>
<td>POSITIVE</td>
<td>10^-1</td>
<td>INCOMPLETE</td>
</tr>
<tr>
<td>BL 3</td>
<td>40</td>
<td>POSITIVE</td>
<td>10^-1</td>
<td>INCOMPLETE</td>
</tr>
<tr>
<td>BL 4</td>
<td>40</td>
<td>POSITIVE</td>
<td>10^-2</td>
<td>COMPLETE</td>
</tr>
</tbody>
</table>

** INOCULATION WITH L.pomona BACTERIN

** HIGHEST DILUTION SHOWING AT LEAST 50% AGGLUTINATION-LYSIS

Figure 5

CROSS ABSORPTION OF Br. abortus AND L.pomona POSITIVE SERA

<table>
<thead>
<tr>
<th>CALF NO.</th>
<th>DAYS* POST INOC.</th>
<th>END POINT TITER-AFTER ABSORPTION WITH LEPTOSPIRA</th>
<th>LEPTOSPIRA REACTION</th>
<th>DILUTION</th>
<th>BRUCELLA REACTION</th>
<th>DILUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 1</td>
<td>40</td>
<td>POSITIVE</td>
<td>INCOMPLETE</td>
<td>1 : 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL 2</td>
<td>40</td>
<td>POSITIVE</td>
<td>COMPLETE</td>
<td>1 : 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL 3</td>
<td>40</td>
<td>POSITIVE</td>
<td>COMPLETE</td>
<td>1 : 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL 4</td>
<td>40</td>
<td>POSITIVE</td>
<td>COMPLETE</td>
<td>1 : 25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* INOCULATION WITH L.pomona BACTERIN.

Figure 6

DISCUSSION

The development of Brucella agglutinin titers by six of eight calves infected with L. pomona suggested an antigenic relationship between the two organisms. However, reciprocal absorption of these six sera with Brucella and Leptospira antigens did not affect the heterologous agglutinin
titers. Homologous agglutinins were not detectable following these absorptions. This indicates that the agglutinins were produced in response to separate antigens.

There are several possible explanations for the Brucella agglutinins developed by groups LO and LW. It has been documented that an antigenic relationship exists between Pasteurella and Brucella organisms. It is possible that antibodies for Pasteurella organisms which were present in the body or environment of the calves, might be anamnestically stimulated by infection with L. pomona resulting in the low Brucella titers seen. These calves did not show symptoms of pasteurellosis at any time. The Brucella agglutinins developed in groups LO and LW might also have been of the non-specific type isolated and purified by Rose and Roepke. The results of the acidified plate antigen and heat inactivation tests support the latter view.

The results of the other phases of this experiment also tend to support the theory that these were non-specific antibodies and argue against the theories of anamnestic response or serological relationships between the organisms. The calves injected with Br. abortus strain 19 developed high Brucella titers, but failed to develop demonstrable leptospiral agglutinins. The group BL calves were used to prepare sera containing antibodies against both etiological agents. Absorption of these sera with either antigen reduced the homologous serum titer to a negative reading while the heterologous serum titer remained unaffected. Three successive inoculations of the Brucella vaccinated calves with L. pomona bacterins failed to produce an increase in their Brucella serum agglutinins. This evidence argues against the theory of anamnestic response.

CONCLUSIONS

The evidence gained from reciprocal absorptions indicates that L. pomona and Br. abortus did not contain common antigens or cross reacting antigens under the conditions of this experiment.

Evidence of a serological relationship between L. pomona and Br. abortus was not found. The authors are of the opinion that the Brucella serum agglutinins which developed in six animals of groups LO and LW were non-specific in nature, possibly of the type described by Rose and Roepke.

Repeated vaccination with L. pomona bacterins did not produce a rise in the Brucella titer of animals so treated.

REFERENCES


RECENT ADVANCES AND NEEDS FOR FUTURE EMPHASIS
IN RESEARCH ON LEPTOSPIROSIS

Mildred M. Galton, Sc.M.

Although the epidemiology of leptospirosis has been shown to follow a characteristic pattern, recent knowledge suggests that the methods of spread of the disease are influenced by a complexity of variable conditions. A wide range of domestic and wild animals have been found infected with leptospires. After acute, mild, or more frequently, inapparent infection, the organisms become established in the kidney and are eliminated in the urine. Transmission of leptospiral infections to man and other animal hosts results from direct or indirect contact with the urine of these animal carriers. The organisms usually enter the body through the oral, nasal and conjunctival mucosa, or skin lesions. It is believed, also, that entrance may be through unbroken skin softened by long exposure to water.

The spread of infection depends largely on favorable environmental conditions of humidity, rainfall, temperature, sunlight, and the pH of water and soil, which affect the duration of survival of leptospires. Several investigators have studied the effects of pH; and, although differences exist in the response of serotypes and strains, it is generally agreed that all leptospires survive longer in alkaline or neutral waters. However, Gordon-Smith and Turner studied the survival of four leptospiral serotypes in buffered distilled water at pH range from 5.3 to 8.0. They found that at pH below 7.0, survival ranged from 10 to 117 days and at pH above 7.0, from 21 to 152 days. In aqueous extracts of soil samples from different areas in Malaya, these authors found no correlation between pH and survival time.

In Queensland, a soil sample collected on a cane farm and sent to a laboratory 1100 miles away, was believed to be the source of leptospiral infection in the scientist who examined the sample. The soil was moist and of pH 5.5. This incident illustrates the risk of handling soil samples without gloves.

Dutch investigators observed that the incidence of leptospirosis due to bathing and accidental immersion, was greatly reduced when the salinity of the Amsterdam canals was raised by flooding with sea water. That other factors may play an important role was suggested during the Malayan studies by a low incidence of leptospirosis in workers in some of the rice fields where a high rate of infection existed in the rodents. As the phenomenon could not be explained by adverse pH or salinity, the soil was studied. Bentonite clay similar to the montmorillonite clay of the

From the Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Epidemiology Branch, Veterinary Public Health Laboratory, Atlanta 22, Georgia.

Presented at the 67th Annual Meeting of the U.S. Livestock Sanitary Association, Albuquerque, New Mexico, October 17, 1963.

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rice fields, was found in a series of experiments to absorb about half the leptospires from suspension. It has been further demonstrated that certain bacteria discourage the survival of leptospires while others have little or no effect. Recently, Kirschner observed that certain strains of staphylococci and sarcina actually promoted the growth of virulent leptospires in vitro. These findings lead one to wonder whether this symbiotic phenomenon may occur, also, in nature.

The almost universal seasonal prevalence of outbreaks of human leptospirosis during the warm, humid months, supports the view that climatic conditions affect leptospires. Obviously, the dilution factor is important, also, in the maintenance of leptospires in the environment. In experimental studies with serotype pomona, Okazaki and Ringen detected this organism in water-saturated soil after 183 days, while in damp soil, it could be found for three to five days, and in dry soil for only 2-1/2 hours. The concept that water must be stagnant or in slow moving streams for leptospires to survive, no longer holds true. Gillespie et al., have recovered serotype pomona from minimal amounts of water from a stream estimated to be flowing at a rate that would result in a very high degree of dilution within a 24-hour period.

Assuming all factors are optimum for survival of leptospires, the prevalence of human infection acquired from a contaminated environment would probably depend upon the number of carrier animals and the number of leptospires these animals are shedding. Unfortunately, this is not always true, as pointed out by Gordon-Smith and Turner in their failure to detect leptospirosis in Malayan rice field workers when conditions for transmission appeared favorable. Their work suggested the involvement of certain types of soils and, possibly, chemical factors. For this reason, further studies, particularly on soils, are needed to clarify this problem.

Differences not only in the occurrence and distribution of various serotypes throughout the world but in the range of clinical manifestations produced by the same serotype in animals in certain areas, present more perplexing problems. Larson recently commented that wherever a clinician who was aware of leptospirosis went in the United States, cases were found; when the clinician moved, no more cases appeared in the area. Obviously, medical and veterinary clinical awareness and the availability of laboratory diagnostic aid, are important and necessary in our attempts to interpret and understand differences in distribution of serotypes in man and animals.

In the United States, leptospiral serotype pomona occurs commonly in cattle and produces both severe and mild infections within the same herds; neurologic manifestations are rare. This serotype is found frequently in wildlife, particularly skunks. Human infections characterized by meningeal symptoms also, are reported frequently. Similar manifestations occur in human infections with serotype pomona of swine origin in Central Europe, but these signs rarely occur in infections by this serotype among Italian rice field workers where the source is believed to be rodents.

In Israel, bovine leptospirosis is a serious problem. Severe and fatal infections are also frequent in goats, but infection with serotype
RESEARCH ON LEPTOSPIROSIS

pomona has been reported only rarely in imported cattle. Grippotyphosa and canicola are the serotypes involved in both domestic animal and human infections. A local vole, Microtus guentheri, appears to be the main host of serotype grippotyphosa, and natural reservoirs of serotype canicola include not only the domestic dog, but jackals and hedgehogs.

In the United States, serotype canicola is a frequent cause of infections in dogs and man, and it has been isolated from skunks. Infections in cattle and swine have been detected only rarely. The distribution of serotype grippotyphosa in the United States is also quite different from that in Israel. This type has been reported only in raccoons, skunks, and foxes. Occasionally, serologic evidence has suggested infection in man, cattle, and deer.

Many more examples of these unexplained differences in serotype distribution could be cited. They serve merely to point out our lack of knowledge of the various factors that contribute to the type of disease manifested by a specific leptospiral serotype in a particular host in different areas or periods. Certainly, area and time must be considered along with other factors in developing the concept that each leptospiral serotype usually has a primary animal host.

The relationship between the nature and outcome of leptospiral infection, the serotype, and the host animal is not always clear. The same dose of similar organisms may produce fatal infection in some species of animals, while other animal species may show no signs of illness, but become renal carriers. Faine studied factors affecting the development of the carrier state in leptospirosis in experimentally infected mice. Growth curves of young mice were of two distinct patterns: a "lethal" pattern of continued weight loss until death, or a "carrier" pattern of retardation of weight gain showing a distinct lag phase. The numbers and virulence of the infecting leptospires, and the age of the mouse determined the outcome of the infections. Two other interrelated variables that influence the outcome of infection include the susceptibility of the host animal and the serotype of the infecting leptospires. Faine concluded that the "host of election" hypothesis is tenable if it is regarded as the result of a quantitative rather than qualitative adaption of host and parasite to one another. Further studies on the carrier state in mice indicated that this condition may be attributed to the ability of virulent leptospires to a) grow in the host and produce lesions and the primary, acute generalized infections and b) grow in renal tubules in the presence of antibody.

OCCURRENCE OF LEPTOSPIRAL SEROTYPES IN THE UNITED STATES

Cattle, Swine and Dogs:

Since leptospires were first isolated from cattle in New Jersey in 1948 and later identified as serotype pomona, these infections, characterized by acute to inapparent disease, have been recognized with increasing frequency throughout the country. In 1954, the United States Department of Agriculture, Agricultural Research Service, estimated that annual losses were over a hundred million dollars. By 1960, evidence
of the disease was reported from all States except Alaska. In 1961, a communication from Dr. O. J. Hummon, Laboratory Services, Animal Disease Eradication, United States Department of Agriculture, suggested that acute bovine leptospiral infections may be decreasing. He stated that serologic surveys (Stoenner plate test) were conducted in various States in cooperation with Federal-State brucellosis laboratories. During a 4-1/2 year period (January 1957 to June 1961), more than a million cattle from over 68,000 herds were tested. From these, the herd infection rate was found to be 4.21 percent, and the cattle infections were 0.44 percent. In these tests, only antigen of serotype *pomona* was used. During a slightly earlier five-year period (1955-1959) in Alabama, more than 7000 cattle sera were tested by the microscopic agglutination test with live antigens. For most of the samples, six leptospiral serotypes were used: *pomona, sejroe, autumnalis, canicola, grippotyphosa* and *icterohaemorrhagiae*. Antibodies against serotype *pomona* were detected in 33 percent of these sera, against serotype *sejroe* in 35 percent, and against the remaining four serotypes in four to eight percent. Serotypes *pomona* and *canicola* were isolated from cattle. The authors suggest that an increase in positive samples during the last two years of the period may be attributed to more selective sampling, since practitioners were more familiar with the signs of the disease. Although serotype *sejroe* has not been isolated from cattle in the United States, several strains of a closely related serotype, *hardjo*, have been obtained in Louisiana, Nebraska, and Pennsylvania, from animals both with and without clinical signs of disease.

From an epizootiological point of view, leptospirosis in swine probably constitutes a greater and more important public health hazard than the disease in cattle. Infected pigs apparently shed the organisms in their urine in greater numbers and for longer periods of time than do cattle. Abortion, birth of weak pigs, metritis and lowered fertility are frequently the only signs of infection. The disease actually may be more widespread in swine than in cattle. Again, the primary infecting serotype appears to be *pomona* although *canicola* was isolated from swine for the first time in Columbus, Georgia, during the study of an outbreak involving 26 human cases. In Scotland, Coghlan *et al.*, established that apparently normal pigs were harboring serotype *canicola* and were responsible for outbreaks among farm workers. Their observations indicated that the infection in a herd of pigs may be maintained for many years.

It has been estimated that 25 to 35 percent of the dogs in this country have or have had leptospirosis. In a recent study of 156 stray dogs in Florida, White *et al.*, isolated leptospires from 17 (11 percent). Sixteen of these cultures proved to be serotype *canicola* and one was serotype *icterohaemorrhagiae*. Leptospiral antibodies were detected in the serum of 24 percent. In another study, antibodies were detected in 35 percent of sera from 357 normal dogs from Alabama. While *canicola* is the predominant serotype in dogs, serotype *pomona* has been isolated occasionally, and antibodies have been found against at least four other serotypes. Inapparent infections appear to be frequent in dogs as evidenced by a recent investigation in the Communicable Disease Center, Veterinary
RESEARCH ON LEPTOSPIROSIS

Public Health Laboratory, of 20 dogs in a local kennel. During ten months following the occurrence of a fatal case, serotype canicola was isolated from the urine of nine dogs, none of which showed the slightest sign of illness. Urinary shedding may occur for weeks or months after infection and when antibodies cannot be detected. Many cases and outbreaks in domestic animals can be traced to direct or indirect contact with infected animals in the same herd or kennel; others often cannot be explained.

Wildlife:

Within recent years, a variety of leptospiral serotypes has been found in many wildlife species in widely separated areas of the United States. Attention cannot be focused on rodents alone, but all warm blooded wild animals, as well as birds, reptiles and turtles, must be considered as potential hosts.

Extensive investigations of wild animals as possible hosts of leptospires in the United States were commenced in southwest Georgia in 1953 at the Communicable Disease Center Field Station, at Louisiana State University in 1957, and at the University of Pennsylvania, New Bolton Center, in 1959. During these studies, leptospires were isolated from five to 51 percent of the wild mammals cultured, including skunks, raccoons, opossums, foxes, wildcats, beavers, nutrias, rabbits, armadillos, woodchucks, voles, shrews, mice and rats. Although identification has not been completed on all leptospiral isolates obtained in these investigations, at least 14 serotypes or subserotypes have been found, representing the following groups: ballum, canicola, icterohaemorrhagiae, pomona, autumnalis, australis, grippotyphosa, hebdomadis, bataviae, and hyos. Several new serotypes or subserotypes have been isolated in the hyos group, serotype atlantae and subserotype bakeri, in the hebdomadis group, subserotype georgia; and in the pyrogenes group, subserotype myocastoris. The highest incidence of infection has appeared in the striped skunk, and the next highest in opossums and raccoons. Skunks, also, have been found to harbor the greatest number of serotypes.

Limited epizootiological studies were undertaken in North Carolina in the area of the now historical Fort Bragg fever outbreaks. Serotype autumnalis, implicated in this outbreak, was not found, but two serotypes, australis and grippotyphosa, were isolated from raccoons and foxes. In addition, investigations of wildlife hosts on farms where leptospirosis existed in domestic animals or in range areas, have been conducted in Maryland, Pennsylvania, Illinois, Ohio and Oregon. With the exception of Oregon, these studies confirm the widespread distribution of leptospirosis in our wildlife.

With the increase in the deer population observed in recent years in many areas of the United States and a lack of knowledge concerning prevalence of leptospirosis in deer, investigators in some of the southeastern, northeastern and middle-western States have directed their attention toward this possible wildlife reservoir. The microscopic agglutination test with a battery of six to 12 leptospiral antigens has been used in
several extensive serologic surveys; in others, only antigen of serotype *pomona* was used. Leptospiral antibodies have ranged from zero in some sections, to 20 percent in others. The majority of the positive reactions were to serotype *pomona*; but, in areas where other antigens were used, reactions with antigen of serotype *grippotyphosa* occurred frequently. To a lesser degree, titers of antigens of serotypes *sejroe*, *canicola* and *icterohaemorrhagiae* have been observed. The only serotype isolated from deer, thus far, has been *pomona* in New York State and Ontario. Clinical disease has been produced in deer and the organisms recovered by experimental inoculation of a strain of serotype *pomona* freshly isolated from cattle. In some areas, deer commonly graze with cattle; but there is, as yet, no firm evidence as to whether deer are disseminators of infection or are victims.

The possibility that birds, reptiles, and other cold blooded species are disseminators of leptospiral infections, has received limited attention. Both field and experimental investigations indicate that fowl and reptiles can be infected with leptospires, but their role in the epizootiology of leptospirosis has not been determined. In Washington State, *pomona* antibodies were detected in sera from chickens on a ranch where active bovine leptospirosis existed. The use of one- to two-day-old chicks as experimental animals has been tried; but, even at this age, natural resistance to infections is evident. The isolation of serotype *lataviae* from the kidneys, liver, heart, and intestine of four species of wading birds captured in the rice fields of Italy, stimulated further studies. Russian investigators observed titers to *pomona* antigen in domestic geese that had had contact with water in an area where infections with this serotype were occurring in man and cattle. In another Russian survey, agglutinins to serotype *saxkoebing* were found in sera from a wild goose.

Investigators in Malaya detected leptospirosis in 41 percent of 37 sera from snakes, in one of 173 bird sera and in one of 70 lizard sera. More recently, Ferris and associates, in Illinois, isolated serotype *ballum* from the liver and kidney of a hog-nosed snake (*Heterodon platyrhinus*) caught in a mouse trap in a cattle shed. In Florida, White found 38 (24.5 percent) of 138 snake sera agglutinated with antigens of one or more leptospiral serotypes in the macroscopic agglutination test. Eighteen (11.6 percent) of the sera reacted in a dilution of 1:50 or above in the microscopic agglutination test with live antigens. Reactions in both tests were observed most frequently with antigens of serotype *ballum*. Attempts to isolate leptospires from liver and kidney tissue were unsuccessful.

The detection of titers in sera from all of 58 water turtles (*Clemmys caspica*) ranging from 1:50 to 1:32,000 in agglutination tests with antigen of serotype *ballum* by Van der Hoeden et al., in Israel, suggests the need for further investigation in this and other species of turtles. Low titers were observed also for serotype *hyos* in 15 of these sera, but negative results were obtained with antigens of 11 other serotypes of pathogenic strains and with four saprophytic water strains. However, all of the
serums agglutinated a fifth water strain, *biflexa*, Gent, to approximately the same titer as serotype *ballum*. No leptospires were isolated from direct cultures from the liver, spleen, and kidneys of these turtles or from hamsters inoculated with these tissues. Further investigations conducted by the authors suggested that the agglutinating factor in *Clemmys* serums is based on a principle different from that of the immune antibodies. Later serums from 12 of the snakes in the Florida study that reacted with antigen of serotype *ballum* were examined in our laboratory by the microscopic agglutination test with live antigen of the *biflexa* strain, Patoc I and another water strain isolated from a river in Iowa, with negative findings.

A recent experimental study has demonstrated the susceptibility of goldfish to leptospires and their ability to harbor these organisms for at least 17 days while living in water with pH 5.0. The leptospires penetrated into the goldfish through the gills which, with the kidneys, seemed to be the main sites of localization of the organisms. These observations suggest that fish may play an important role in the epidemiology of leptospiral infections possibly as maintenance hosts in rivers and streams. Certainly, these findings and the observations on reptiles warrant further investigations to clarify their position in the chain of transmission.

The role of arthropod vectors in the transmission of leptospirosis has been studied both experimentally and in nature. Several investigators have been able to infect ticks by allowing them to feed on infected guinea pigs or hamsters. These experimentally infected ticks transmitted the disease to normal animals. The isolation of serotype *grippotyphosa* from the European tick, *Dermacentor marginatus* S., has been reported from Russia. The ticks were from cattle where leptospirosis had occurred among the herd. More recently, Van der Hoeden has isolated serotype *canicola* from *Rhipicephalus sanguineus* collected from a hedgehog. However, under natural circumstances, they attach no great importance to the transmission of leptospirosis by ticks in Israel. Recent experimental studies in Poland suggested the presence of a leptospirocidal substance in *Ixodes ricinus* ticks. This observation may account, in part, for the fact that ticks have not been found to be important vectors. Further work should be undertaken to determine the nature and location of the leptospirocidal substance in these ticks and whether the substance is present in other species of ticks.

**OCCURRENCE OF HUMAN LEPTOSPIROSIS IN THE UNITED STATES**

Reports of human leptospiral infections in the United States are appearing more frequently. This may be due, in part, to greater clinical awareness and the availability of laboratory diagnostic service. During the past 16 years, with minor exceptions, there has been a rather steady increase in the number of cases of leptospirosis reported to the National Office of Vital Statistics or to the Communicable Disease Center for inclusion in the Annual Supplement of Morbidity and Mortality Weekly Reports (Figure 1). A total of 715 cases have been reported ranging from a low of 14 in 1947, to a high of 79 in 1962. Reports of cases are usually
Received annually, although the recommended reporting requirements in the 1960 edition of the American Public Health Association's Handbook on Control of Communicable Diseases in Man, place leptospirosis in the class of diseases on which "case report is regularly required, by most practical means; forwarded to next superior jurisdiction as a collective report, weekly by mail." In some instances, however, cases are not reported through this official channel. For example, in 1962, 79 cases were reported officially. Findings on serum samples referred to the Communicable Disease Center for serologic testing for leptospiral antibodies, plus clinical and epidemiologic data, indicated infections in at least 11 additional patients that were not included among the officially reported cases.

Between 1950 and 1961, 144 deaths attributed to leptospirosis were reported. This represents a death to case ratio of 24 percent on the basis of the 590 cases reported for those 11 years. This apparent high fatality rate, undoubtedly, is due to lack of recognition of many benign infections. In contrast, during 1962, only two (five percent) of 40 cases were fatal on which serological, clinical, and epidemiological data was obtained by the Communicable Disease Center and Walter Reed Army Institute of Research.

More than half of the outbreaks reported in this country, have been attributed to immersion of the individual in water contaminated by cattle or swine infected with serotype *pomona*. Sporadic cases among certain occupational groups, such as abattoir and dairy workers, with a history of direct contact with cattle and swine, have been recognized with increasing frequency.
A serologic investigation for evidence of leptospiral infection in dairy farmers, cattle ranchers, and packing house workers, was reported recently from Nebraska. The highest percent (8.8) of antibodies was found in sera from 34 packing house workers. Dairy farmers were next with 3.8 percent of 202 appearing positive, and the lowest group positive were the cattle ranchers, 2.6 percent of 76. It would be expected that the first two groups would have the closest direct contact with infected animals. Tobie and McCullough found antibodies against serotype pomona in the serum from 28 (11.3 percent) of 246 meat inspectors in packing houses in New York, Baltimore, and Chicago areas. Earlier studies on 233 packing house workers in Omaha, revealed that 6.4 percent of this occupational group had antibodies for serotype pomona.

LABORATORY DIAGNOSTIC AIDS

Some mention should be made regarding recent developments in laboratory aids that may be useful in epidemiological and epizootiological investigations. It is well established that leptospires may be isolated from the urine of dogs and other animals, particularly rodents, when antibodies cannot be detected in their serum. For example, homologous antibody could not be detected in 16 sera from raccoons, opossums and skunks, found to be infected with subserotype georgia; and no reaction was obtained when two of these raccoon sera were tested against 16 other members of the hebdomadis group. Therefore, the limitations of serologic tests to detect leptospiral infections, particularly in carrier animals, are obvious. Serologic tests do have value as a screening tool to detect the presence of infection in an area, but these tests should always be supported by culture studies to establish the infecting serotype and to determine its prevalence. Serology is also valuable when paired specimens on suspected human and animal cases can be examined to detect a rise or fall in titer.

In the United States, the established presence of at least 14 serotypes or subserotypes in man or domestic and wild animals, plus a new pathogenic type from water, dakota, a subserotype of serotype naarm, emphasizes the need for multiple antigens or a genus specific antigen in testing. In our experience, the rapid macroscopic slide agglutination test with 12 formalin-killed antigens, has been most satisfactory. It has been found that in some cases, antibodies can be detected earlier in the course of disease by this test than by the microscopic agglutination test with live antigens.

Studies on a genus specific antigen have been tried recently in Europe. Sturdza et al. used the complement fixation test with an antigen prepared from the Patoc I strain of biflexa, in the examination of 900 human serum samples. Of these, 170 samples were positive, representing 153 cases of leptospirosis. One hundred thirty-eight (89 percent) of these cases were confirmed by the conventional agglutination procedure. Buddhaieri and Addamiano used a biflexa antigen in both the agglutination test and the complement fixation test, and observed little difference in
sensitivity and specificity of the two tests. They found that the Sao Paulo strain of serotype biflexa reacted best with animal serum, and the Patoc I strain with human serum. Further studies are needed on such genus specific antigens which show broad crossing with pathogenic leptospires and may be useful for screening. The infecting serotype cannot be determined with certainty unless it is isolated and identified serologically.

A dilution method for direct culture of voided urine, developed in the Veterinary Public Health Laboratory, has been successful in eliminating contamination and is practical for field application. The dilution method is useful, also, for direct culture of kidney suspensions.

Some investigators have believed that the use of laboratory animals for the isolation of leptospires, offered no great advantage over direct culture of tissues and body fluids. However, a recent study by Alexander et al., indicates that presently used media cannot supplant, entirely, the use of laboratory animals for the isolation of leptospires. They reported the isolation of a pathogenic leptospiral strain in the icterohaemorrhagiae group, serotype naum, subserotype dakota, from river water that could not be cultivated in conventional leptospiral media. After trials in numerous variations and modifications of the conventional media, growth of the strain was obtained in Fletcher's medium modified by the addition of 20 percent rabbit serum. Apparently, the concentration of serum was not the only critical growth factor in the modified Fletcher's medium, as growth was not obtained in other basic media modified similarly. Difficulties were encountered earlier in the isolation and maintenance in conventional culture media, of occasional strains of serotype hardjo from cattle and members of the hyos and grippotyphosa groups from wild mammals. Obviously, the currently employed media are not suitable for the isolation of strains that possess certain fastidious growth characteristics. Additional study should be undertaken to determine the suitability of the modified Fletcher's medium for routine use.

A more recently applied tool has been the fluorescent antibody technique to stain leptospires in infected urine. Although only limited studies have been undertaken, reports indicate that the method may be a valuable and practical tool for detection of carrier animals. The urine is formalinized and may be sent to a central laboratory through the mail. Good correlation has been observed between findings obtained on samples from the same cases, by the fluorescent antibody method applied to formalin-fixed tissues, and by isolation, serological and silver impregnation procedures. Other fluorescent antibody techniques have been tried to detect leptospiral antibody in serum from suspected cases. Limited studies with both the indirect, and the complement fixation methods, are still inconclusive. Further work is needed to adapt and evaluate these methods for the detection of leptospiral antibodies.

PREVENTIVE MEASURES

In the prevention of leptospirosis, the application of strict sanitary practices plays an important role. Protection of drinking water supplies
for domestic animals from contamination; adequate drainage of wet, muddy farm areas; avoidance of overcrowded feed lots; and rodent control are particularly valuable measures. The use of potentially contaminated waters for swimming by man should be avoided and protective clothing, such as gloves and boots, should be worn by workers in occupations of high risk. Chlorine disinfection of water of swimming areas should be carried out wherever possible. Cleaning barns and work areas in poultry and other food processing plants with disinfectant agents, as a sodium hypochlorite solution, effectively eliminates contamination. Disinfection of fields with copper sulfate or calcium cyanamide has been tried, but results have been successful only under limited conditions.

Little progress has been made in developing a practical method for elimination of the "shedder" state in animals. Antibiotic treatment (dihydrostreptomycin and tetracyclines) has been recommended by some investigators, but the high cost of antibiotics usually limits their use to individual animals. Further studies are needed to develop effective and practical procedures for eliminating the carrier state.

Leptospiral vaccines have been reported to be successful in agricultural workers in Japan and several European countries. Some vaccines, however, have caused severe side-reactions. The most encouraging results have been obtained in Italy where a formalinized antigen was used in rice field workers. In the United States, vaccines have not been used in man; but, with the widespread distribution of leptospirosis in our domestic and wild animals, consideration should be given to the possibility of vaccinating certain occupational groups. In contrast, vaccines are being used frequently in this country in cattle and swine (prepared with serotype pomona) and in dogs (prepared with serotypes icterohaemorrhagiae and canicola), but their effectiveness is difficult to evaluate. Protection against clinical illness caused by these serotypes has been reported for varying periods up to 20 months, but vaccines do not protect against the development of the carrier state.

Robertson and Boulanger used guinea pigs to evaluate seven commercial bacterins of serotype pomona prepared for use in cattle. They observed a wide variation in their antigenicity and in their ability to provide protection. These authors emphasized the importance of using multiple criteria to evaluate the efficacy of a product.

The development of acute anaphylaxis in cattle has been attributed to sensitization by antigens of serotype pomona. Morter et al., obtained evidence which suggested involvement of a leptospiral cell component rather than rabbit serum. These anaphylactic reactions have occurred frequently among animals revaccinated at intervals of less than one year. Further study of this problem is needed. In addition, carefully controlled studies should be carried out on vaccines for cattle and swine which will provide firm evidence of their value.

Kemenes has studied the cross immunity of pathogenic leptospires of different serotypes in guinea pigs. Cross immunity was observed against serotypes icterohaemorrhagiae, canicola, and pomona, and to a lesser extent, against serotype grippotyphosa. Such immunity was not
observed against serotypes hyos and sejroe. He interpreted his findings as suggesting that this immunity takes place only with leptospiral types with the same biological properties, and that it is not based on an antigenic relationship.

INTERNATIONAL COOPERATION

National and international cooperation in reporting and exchange of information on the epidemiology, occurrence, pathogenesis, preventive and therapeutic measures, and laboratory methodology, is essential to correlate the many puzzling factors posed by this disease. Excellent progress in this respect is being made. From a nucleus commenced in Schuffner's and Wolff's laboratory in Amsterdam in 1923, it has been extended throughout the world. In 1952, the WHO/FAO recognized the need for consideration of the public health aspects of leptospirosis. This organization established a leptospirosis study group and, subsequently, designated reference laboratories to function as expert centers for research for different regions of the world. The functions of these laboratories were to supply leptospiral cultures and specific antisera to national and other recognized laboratories working in the field of leptospirosis research in their region; to assist these laboratories, when possible, in identifying problem strains; and to accept individuals for training in leptospiral methodology for limited periods. The Taxonomic Subcommittee on Leptospira of the International Committee on Nomenclature is active, also, in stimulating international cooperation. In February 1962, WHO convened a meeting of the Scientific Group on Research in Leptospirosis at Geneva. The Taxonomic Subcommittee reviewed the report of this group to the Director-General and recommended that it be published. In the United States, a Leptospirosis Research Conference, established in 1958, meets annually following the Animal Disease Research Workers meeting in Chicago. This two-day meeting provides an opportunity for exchange of ideas and current information between research workers in this country.

SUMMARY

In summary, the methods of spread of leptospirosis are influenced by a complexity of variable conditions, as pointed out in the beginning of this report. Some of these methods follow a basic pattern and are well understood; others remain unexplained. Comparative studies of the prevailing factors in areas with different climates and environmental conditions, should provide valuable information, leading toward a better understanding of some of the unexplained problems of leptospirosis.

Further evidence is needed to establish the actual role of the many wild animal hosts in the chain of transmission. Are raccoons, skunks, opossums, deer, and other wild animal hosts, a problem as disseminators of leptospiral infections among our domestic animals and man, or are they, themselves, victims of infection through contact with contaminated farm areas and infected rodents? What role is played by birds, reptiles, and fish in the maintenance of leptospiral organisms?
Establishment of immune domestic animal populations by vaccination, use of disinfectants to destroy leptospires in certain contaminated strategic areas, and education of the human population (particularly hunters) regarding the danger of infection through contact with infected urine or tissue of these wild creatures, will appreciably reduce the hazard from such foci. In addition, our efforts should be directed towards the development of better screening procedures to detect infection; use of direct culture and animal inoculation techniques to isolate the leptospires so that the infecting serotype may be determined; development of effective prophylactic agents, particularly trials to indicate effect of vaccines on the carrier state; and finally, education of the public as to the necessary hygienic practices.

REFERENCES


77. Clark, L. G.: Personal communication, 1961, University of Pennsylvania, Research studies on leptospirosis in Managua, Nicaragua.
RESEARCH ON LEPTOSPIROSIS


REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

M. J. Twiehaus, Lincoln, Nebraska, Chairman; E. H. Bohl, Columbus, Ohio; R. J. Byrne, Silver Spring, Maryland; D. E. Hughes, Ames, Iowa; S. G. Kenzy, Pullman, Washington; A. W. Monlux, Stillwater, Oklahoma; R. L. Morter, Lafayette, Indiana; C. S. Roberts, Auburn, Alabama; L. W. Turner, Nashville, Tennessee

The Committee has reviewed its past reports and recommendations. We found that present viewpoints do not conflict with those previously stated. In this report we propose to emphasize those aspects of leptospirosis most in need of continued attention.

We feel that the lack of detailed morbidity data limits the scope of our recommendations. In the absence of significant incidence data recommendations relative to the use of bacterins or other control procedures are of necessity based upon opinion. For this reason we believe that the Agricultural Research Service should collect and tabulate this data on a national scale. On the field level it is imperative that diagnosis of leptospirosis be confirmed by sound laboratory examinations and not be limited to observation of clinical signs.

Laboratory diagnosis of leptospirosis can be accomplished by existing laboratories using conventional methods of serology, bacteriology, and histopathology. These methods are outlined briefly here as a guide for interested parties.

Several serological methods of detecting leptospiral antibodies are recognized. The agglutination-lysis test (AL) is generally accepted as the most accurate. A detailed description of this test was given in our 1959 and 1960 reports. Macroscopic plate antigens are available commercially. They appear to serve a useful purpose as screening tools or as herd diagnostic tools rather than on an individual animal basis in the cases of Cattle and Swine. The fluorescent antibody technique is being used as a serological tool in several important diseases. Published reports indicate its applicability to leptospirosis. Laboratories equipped for this work should consider utilizing the technique in diagnosis of leptospirosis.

Culture examination of blood, urine, or tissue can be accomplished by any laboratory equipped for bacteriological work. In many instances veterinarians in the field can collect specimens and inoculate culture mediums which can be sent to the laboratory for incubation and subsequent examination. The procedures require only the application of skills and training already possessed by these individuals. A detailed outline of the method of culture was presented in our 1960 report. Further assistance can be obtained from the various members of this committee. Detailed attention must be given to aseptic collection of materials for culture. In this respect the importance of thorough cleaning of the skin and mucous membranes cannot be over emphasized. Inoculation of laboratory animals for detection of leptospirosis is also a useful procedure.
Leptospires isolated by any of these methods should be identified by the recognized serological procedures. Assistance in making these identifications can be obtained from the World Health Organization Leptospirosis Reference Laboratory at the Walter Reed Army Medical Center in Washington, D.C. or the Veterinary Public Health Laboratory at the Communicable Disease Center in Atlanta, Georgia.

Histological examination of tissues may provide an adjunct to serological and cultural methods - formalin fixed heart, liver and kidney tissue stained by silver impregnation methods make possible the visualization of leptospires. This is particularly applicable to fetal tissues since the organisms frequently are not viable.

From our discussions it appears that anaphylactic reactions encountered following revaccination have become less of a problem in recent years. The committee feels that much of the credit for this reduction can be attributed to the efforts made by the biological industry to reduce foreign proteins in their bacterins.

It is evident that additional basic research is needed in the pathogenesis of this disease. Many areas of knowledge about leptospirosis are incomplete or lacking. Until these voids are filled more detailed recommendations pertaining to the control or possible eradication of this disease cannot be made.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this committee continue.
BOVINE MYCOPLASMA MASTITIS*

L. E. Carmichael**, R. S. Guthrie***, M. G. Fincher***,
L. E. Field***, S. D. Johnson*** and W. E. Linquist***

Mastitis associated with *Mycoplasma* was first reported in a brief account by Davidson and Stuart¹ in 1960. These English workers later gave a detailed report of their findings of an extensive outbreak of severe mastitis in an assembled herd.² In 1962 Hale *et al.*³ described a *Mycoplasma* species as the cause of a severe outbreak of mastitis in a Connecticut dairy herd and reported the results of transmission experiments and histopathological examinations of affected udders. The first New York isolation was made in January, 1962, from samples of purulent secretions obtained from nine cows in a Lewis County herd experiencing severe purulent mastitis that had proved refractory to all attempts at treatment. Isolations were then made in February, 1962, from frozen milk samples collected from herds in Cayuga and Tompkins counties. As of August, 1963, *Mycoplasma* have been isolated from a total of 15 herds in New York State, principally from herds in Lewis and Montgomery counties.

The common complaint of the practitioners attending the initial outbreaks was the apparent severe damage to infected udders and the alarming numbers of cows ultimately involved in affected herds and also the sense that a problem not previously seen had arisen.

This report deals with the methods of isolation and the identifying characteristics of *Mycoplasma* recovered from the udder secretions of one or more cows in herds in various parts of New York State, and also records of observations which may prove helpful in recognizing this disease.

CHARACTERISTICS OF THE *MYCOPLASMA*

*Isolation media and method:*

Several media for *Mycoplasma* were employed initially and prepared as described by Barber and Fabricant.⁴ When the organisms proved not to be particularly fastidious, a relatively simple medium then was used, which was composed of the following ingredients:

This report was made possible through the participation of other staff members of the New York State Mastitis Control Program located at various regional laboratories: H. L. Brown, J. B. Cheney, H. C. Temple, R. H. Volgenau, V. L. Boldt, Field Veterinarians; Frances Barnes and Harriet D. Emmette, Research Technicians.

*This investigation has been aided by a grant from the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

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beef heart infusion broth (BHIB, Difco): 89 percent
sterile swine serum: 10 percent
yeast extract (Difco): 1 percent

For primary isolations, penicillin (100 units per ml.) and thallium acetate (0.1 percent) were added to the basal medium to control bacterial growth. After initial isolation was accomplished the bacterial inhibitors were omitted for at least five serial transfers to observe the L-phase bacterial variants. For solid media, beef heart infusion agar (BHIA, Difco) was substituted in the above formula for BHIB. For certain characterizations of the isolates additional media were employed. These included five percent horse blood agar (HBA); phenol red broth supplemented with swine serum and one percent carbohydrate representative of hexose and pentose oligosaccharides, polysaccharides, and alcohols; methylene blue milk; litmus milk; and swine serum agar containing tetrazolium chloride.

For initial isolation from mammary secretions or tissue suspensions, 0.1 ml. samples were pipetted into tubes containing three ml. of serum broth. Then, after three days at 36°C, a drop of broth was placed without streaking onto plates of serum agar. Plates then were incubated in a moist chamber containing approximately 10 percent carbon dioxide. A candle jar containing a moist cellulose sponge was used for this purpose. Plates first were examined after 48 hours and finally discarded if negative after five days.

Growth Characteristics:

Growth in serum broth or on solid media was rapid. After 24 hours slight turbidity was visible in broth cultures and by 48 to 72 hours uniform faintly cloudy turbidity occurred. A fine granular sediment was present in the bottom of inoculated tubes by 48 hours. On solid media growth first could be detected (at 100X magnification) as minute colonies approximately 0.02 mm. in diameter. Colonies reached maximal size after 72 hours. On HBA growth was somewhat slower than on swine serum agar, for maximal colony size was not reached until about four days. All of the isolates caused alpha-type hemolysis on HBA after three to four days incubation in a moist carbon dioxide atmosphere. Hemolysis did not occur in broth media that contained two percent washed horse erythrocytes. Although good growth occurred in aerated broth cultures, carbon dioxide was required for optimum colony formation on solid media. No growth occurred under anaerobic conditions.

Morphology:

Two distinct colonial forms were observed on primary isolation from infective mammary secretions or tissue suspensions (Figures 1 and 2). Colonies were most numerous at the edge of the droplet, however, they could also be seen scattered throughout the film. One colony type was small, coarse, and approximately 0.05 mm. in diameter after three days' growth. This type did not show a distinct dense central disc. The second colony type was distinctly larger in size, measured about 0.1 mm. in
Figure 1. "Small-type" colony of mastitis *Mycoplasma*. Photographed *in situ* from three-day culture of abnormal milk on agar (500 X mag.) Note: lack of discreet centers and rugose surface.

diameter, and showed a dense central disc surrounded by a lighter peripheral zone that was smooth and contained several vacuoles and dark granular elements. On HBA broth types of colonies occurred and each was surrounded by a small zone of partial hemolysis. Hemolysis sometimes appeared complete, but only when the inoculum contained large numbers of organisms. Initially, the colonies were mostly of the "small" type, however, after transfer to fresh media most were the larger type that showed dark centers and light finely granular peripheral zones. Colony morphology varied greatly depending upon the density of organisms in the inoculum and also upon the moisture content of the media. Freshly prepared media, lightly inoculated, consisted predominantly of large colonies with opaque centers.

Microscopic examination of broth suspensions and colonies on agar, fixed by the method of Klieneberger-Noble,\(^5\) showed organisms that ranged in size from coccoid granules that measured about 200 millimicrons in diameter to short branching filaments or cocco-bacillary forms that
varied from 400 to 700 millimicrons in length. Long mycelial elements were not seen. The pleomorphic cocco-bacillary forms were the most commonly observed types in broth and agar preparations, and also in milk films prepared from mastitic udders and stained with Giemsa's stain. The organisms were faintly Gram-negative, but stained well with Giemsa or Macchiavello's stain, where they appeared violet to blue.

**Biochemical Characteristics:**

Carbohydrate fermentation was extremely slow, and of the various sugars and alcohols tested, only fructose and galactose showed slight acid formation, but no gas, after five days' incubation. Glucose, lactose, sucrose, maltose, trehalose, dextrin, glycogen, sorbitol, manitol, dulcitol, and salicin were not fermented after eight days. Methylene blue milk was very slightly reduced after five days, however, tetrazolium chloride was not reduced. Growth, but no coagulation or proteolysis, occurred in sterile skim milk supplemented with five percent swine serum.
Sensitivity to Antibiotics:

In vitro sensitivity to antibiotics was tested by applying impregnated paper discs ("Multidisc" sets 7-24C and 7-24A) on heavily inoculated serum agar plates that contained no bacterial inhibitors. Sensitivity was demonstrated for oxytetracycline, tetracycline, chlortetracycline, kanamycin, and chloromycetin. No inhibitory action was shown by penicillin, streptomycin, dihydrostreptomycin, polymyxin B, viomycin, or neomycin.

Pathogenicity for Laboratory Animals:

No pathogenicity after five serial transfers could be shown for white Swiss mice, rabbits, embryonating hens' eggs, or guinea pigs inoculated by various routes. The organisms caused degeneration of cultured bovine embryonic kidney cells after two to five days incubation. Cytopathic effects (CFE) consisted of cytoplasmic stranding and rounding of cells that would become shrunken with pyknotic nuclei and drop off the surfaces of the tubes. No characteristic changes such as inclusions or multinucleate cells were observed in Giemsa-stained preparations of infected cells, however, the CPE were accompanied by accumulations of much cellular debris. The entire cell sheet usually would not be destroyed completely.

Hemagglutination or hemolysis:

Hemagglutination or hemolysis did not occur when heavy broth suspensions of Mycoplasma were added to 0.5 percent mouse, fowl, cattle, sheep, dog, horse, or human 0 erythrocytes. Incubations were carried out at 4C, 23C, and 36C. As noted previously, partial hemolysis of horse erythrocytes occurred on HBA incubated for several days in a moist carbon dioxide atmosphere. The hemolytic activity was readily separable from the intact organisms by centrifugation or filtration of broth cultures through Seitz EK filters.

Storage of Cultures:

Storage conditions were not specifically studied, however, Mycoplasma retained viability in infective mammary secretions and udder tissues stored for several weeks at 4C and for more than 1 1/2 years when stored frozen at -50C in a mechanical freezer. Broth cultures did not survive more than two weeks at 36C, however, organisms in broth media survived four weeks at 4C. In the lyophilized state, the organisms have remained viable for more than two years when stored at -20C.

Relationship to Other Bovine Mastitis Mycoplasma isolates:

Mycoplasma isolates from New York State herd outbreaks were compared with three strains that have been described previously from natural outbreaks of bovine mastitis. The prototype New York strain was designated Strain "F". The strains that were compared with the prototype strain are as follows:

Strain "56R" which was recovered from the mammary secretions of all quarters of a cow with acute purulent mastitis in
Ithaca, New York, in 1960. This strain initially was believed to have been an hemagglutinating virus, but later was identified as a *Mycoplasma*.

Strain "D" was supplied by Dr. H. H. Hale, University of Connecticut, Storrs, Connecticut, and was isolated during the summer of 1961 from a severe outbreak of mastitis in a commercial dairy herd. A detailed report of the herd outbreak and characteristics of the causal organism and experimental disease has been published.

Strain "W" was isolated from an outbreak of mastitis in Great Britain and was obtained through the courtesy of Dr. Peter Stuart, Weybridge, England. This strain is culturally and biochemically indistinguishable from *M. bovigenitalium* and a complete account of the outbreak and a description of this strain has been reported.

### TABLE I
Comparison of Bovine Mastitis *Mycoplasma* Strains

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<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>F</th>
<th>D</th>
<th>56R</th>
<th>W</th>
<th>F</th>
<th>D</th>
<th>56R</th>
<th>W</th>
<th>Hemolysis</th>
<th>Hemagglutination</th>
<th>DNA Requirement</th>
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<td>&quot;F&quot;</td>
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<td>&quot;D&quot;</td>
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<td>&quot;56R&quot;</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

aHyperimmune serum was prepared against each strain by inoculating rabbits with washed *Mycoplasma* suspensions in graded doses twice weekly for periods of two to four months. Plus (+) indicates inhibition of growth or bizarre colony formation. Minus (-) indicates no effect of serum.
bUnabsorbed serum gave titers of 1:32 - 1:128 with all combinations. No significantly higher homologous reactions occurred.
cAlpha-type hemolysis on HBA plates after three to five days incubation.
dAgglutination of 0.3 percent sheep or bovine erythrocytes.
eRequirement for deoxyribose nucleic acid (DNA) for primary isolation.

Comparisons were made by the growth-inhibition and complement-fixation tests, hemolytic and hemagglutination reactions, and reported nutritional requirements for primary isolation. A summary of the results given in Table I shows that at least three distinct *Mycoplasma* types have been isolated from natural outbreaks of bovine mastitis. Strain "D" from Connecticut was identical in all respects with Strain "F", characteristic of the New York Isolates. Strain "56R", which was isolated from a single cow with mastitis, has not been recovered again from mastitic udders. Strains with properties identical with "56R" have been isolated frequently, however, from calves showing pneumonia and enteritis. Strain "W" from Great Britain was also unique among the isolates studied. We feel that assignment of a species designation to the New York isolate would be premature until after further characterizations are made especially as regards their normal habitat and their relationship to other bovine *Mycoplasma* species.
CLINICAL SIGNS

A typical herd (Wi) which consisted of 35 grade Holstein cows, had purchased milking cows in 1960 from herds with bad records of mastitis control. This herd was infected with *Streptococcus agalactiae*, but suffered relatively little serious damage until several months after the purchased cows were added. During October, 1961, through October, 1962, 17 cows were sold because of badly swollen udders or extreme atrophy of one or more quarters and also because affected cows gave no salable milk.

No acute general febrile symptoms were observed by the owner in this or other *Mycoplasma* infected herds, although cows infected experimentally all showed elevated temperatures to 105.5 F. on the third or fourth day following inoculation. Temperatures of these cows returned to approximately normal within 24 to 96 hours and remain subsequently within the normal range, with occasional periodic elevations that again would return to normal. Nonetheless, no general symptoms of depression or inap-
petence were noted in any animals. The experimental cows showed a marked leukopenia (neutropenia) that lasted up to two weeks. Minimal leukocyte counts of 1800 to 2500 occurred on the third day after inoculation of three cows, the time at which udder edema and fine chunks of pus just appeared in the milk. The mastitis was characterized by a sharp drop in milk production and extremely swollen udders. In some animals the supramammary lymph nodes were greatly enlarged and somewhat firm. The secretion provided a variety of appearances that initially appeared as slightly yellow without obvious clots. However, on standing for a few minutes, a deposit of fine sediment would separate from the turbid whey-like fluid. Some cows showed definitely watery milk with flakes and a few clots, while others, more chronically infected, would produce milk containing large yellow-white caseous chunks, resembling cottage cheese, in a yellow serous exudate (Figure 3) or pus. Milk leukocyte counts varied from 20 million per mm. to more than 100 million. Occasional cows would show secretions tinged with blood and show a pink cast. Typically a cow that produced sixty pounds daily would suddenly cease production and give no more than a few ounces of colostrum-like material. Some cows would be infected in only one quarter and the infection would remain

![Table II](attachment:table_II.png)

*Incidence of Hopeless Mastitis Cases in Herds with Mycoplasma Mastitis*

<table>
<thead>
<tr>
<th>Herds</th>
<th>County</th>
<th>Total Cows in Each Herd</th>
<th>Percent of Cows With Abnormal Milk (Not all due to PPLO)</th>
<th>Cows Sold Because of Mastitis at Time of Outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wi</td>
<td>M</td>
<td>35</td>
<td>95</td>
<td>17</td>
</tr>
<tr>
<td>B</td>
<td>C</td>
<td>22</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>C</td>
<td>T</td>
<td>50</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>H</td>
<td>L</td>
<td>41</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>R.Mu</td>
<td>M</td>
<td>52</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>JFF</td>
<td>M</td>
<td>35</td>
<td>?</td>
<td>1</td>
</tr>
<tr>
<td>RWD</td>
<td>M</td>
<td>60</td>
<td>?</td>
<td>15</td>
</tr>
<tr>
<td>GS</td>
<td>M</td>
<td>48</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>R</td>
<td>L</td>
<td>53</td>
<td>90</td>
<td>41</td>
</tr>
<tr>
<td>M</td>
<td>L</td>
<td>72</td>
<td>?</td>
<td>25</td>
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<tr>
<td>Z</td>
<td>L</td>
<td>37</td>
<td>?</td>
<td>9</td>
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<tr>
<td>S</td>
<td>L</td>
<td>67</td>
<td>?</td>
<td>3</td>
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<tr>
<td>V</td>
<td>L</td>
<td>40</td>
<td>?</td>
<td>14</td>
</tr>
<tr>
<td>N</td>
<td>L</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>J</td>
<td>F</td>
<td>32</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td></td>
<td>644 cows (1 not known)</td>
<td>49.4 percent in seven herds containing 283 cows</td>
<td>197 cows sold from 13 herds containing 594 cows (33.2 percent)</td>
</tr>
</tbody>
</table>

*a Counties: C = Cayuga; F = Fulton; L = Lewis; M = Montgomery

*b Average percent of cows with abnormal secretion in 1962 in new herds on New York State Mastitis Control Program was 16.5 percent, compared to 49.4 percent in seven herds containing 283 cows.
localized, however, most natural and all artificially infected cows developed acute mastitis in all quarters. Curiously, some cows that had not been infused with antibiotics or stanchioned adjacent to infected animals developed mastitis. Table II shows the salvage rate in cows from herds from which \textit{Mycoplasma} was isolated (33.1 percent), a rate which is approximately twice the usual one in herds entering the New York State Mastitis Control Program for the first time. It should not be thought that all cows affected are hopelessly damaged, for although production was diminished, some recovered animals produced reasonably well at their next lactation, following a long period of rest (Table III).

\section*{PATHOLOGY}

\textit{Pathological examination*} was made of tissue blocks taken from various levels of the mammary glands of two experimental cows that had been inoculated two to seven weeks previously. \textit{Gross examination} of affected udders showed swollen glands with the glandular tissue uniformly firm in consistency. The cisternal mucosa and collecting ducts showed nodular or papilliform projections from the surface that were discrete, slightly brown in color, and irregular in outline. These were approximately one to three mm. in diameter. Mucosa of one of the teat cisterns showed diffuse petechial hemorrhages, with a few nodular projections from the surface. In the collecting ducts was a purulent secretion that often contained chunks of insippated pus. The color of the secretion varied from brownish-yellow to pink. Lobulation was distinct. One gland showed a gelatinous area of edema approximately six inches in diameter on the posterior-ventral surface. The supramammary lymph nodes of two cows were approximately three times normal size, and were firm and edematous. The nodes from the third cow were only slightly enlarged. \textit{Microscopic examination} showed in all glands degrees of chronicity, ranging from acute purulent

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Cow & Age & PPLO & Recovering \tabularnewline & & 1961 & 1962 & 1963 \tabularnewline \hline
C - 7 & 19,000 & 10,883 & 8,410 \tabularnewline E - 6 & --- & 14,720 & 10,200 \tabularnewline F - 4 & --- & 6,337a & 11,580 \tabularnewline J - 4 & --- & 8,943 & 11,770 \tabularnewline M - 7 & 13,720 & 9,418 & 10,120 \tabularnewline \hline
\end{tabular}
\caption{Herd Wi}
\end{table}

\textsuperscript{a}Part of year

*Pathological examination of udder tissues was performed by Dr. K. McEntee, Department of Pathology and Bacteriology, New York State Veterinary College, Cornell University, Ithaca, New York.
mastitis to chronic changes with inspissated secretions in the alveolae, with lymphocytes, plasma cells, macrophages, and eosinophils predominating in the interstitial tissue. Neutrophiles predominated in alveolae. As noted by Hale, a common feature in all glands was formation of granulomas at all levels of the gland. There was considerable hyperplasia of the duct epithelium which was accompanied in more chronic areas by an increase of interstitial fibrous tissue. The condition is therefore pathologically a purulent interstitial mastitis with granuloma formation as a common feature.

The supramammary lymph nodes showed cortical hyperplasia. The lymph follicles were active and there was a marked increase in plasma cells, especially in the medullary areas. The sinusoids contained large numbers of macrophages, a few eosinophiles, and neutrophiles which appeared to be draining into the node rather than produced in the sinuses. The medullae were edematous.

Giemsa stains of smears from fresh tissue blocks from various areas of the glands showed myriads of typical organisms. Organisms also were found, by culture and fluorescent antibody methods, in the enlarged lymph nodes and gelatinous subcutaneous edema fluid.

**EPIZOOTIOLOGY**

Factors that were common to the majority of outbreaks observed in New York State included the appearance of *Mycoplasma* mastitis in cows that were housed predominantly in poorly ventilated stables where management practices to prevent any type of udder infection were poor to bad. In addition, over half of the herds that experienced *Mycoplasma* mastitis had purchased milking cows from public sales or from a variety of sources within a few weeks or months before serious unusual mastitis developed. The majority of the herdsmen had treated mastitis cases by udder infusion, using a common syringe and often even a common cannula. These individuals had generally not enlisted the help of the NYSMCP, or had not followed instructions regarding the prevention of mastitis suggested under this Program.

During the period of several severe outbreaks of *Mycoplasma* mastitis in one area, samples of milk were collected from clinically normal herds in this area and also at random on a complete herd basis from herds representing other central New York dairying areas, and who regularly submit to periodic sampling for bacteriological tests by the NYMCP laboratories. Composite aliquots of quarter samples from every fifth cow from a series of 2500 cows participating in the Program were tested for *Mycoplasma*. None of the herds had complained of serious mastitis problems and very little treatment had been performed by the attendants or herd veterinarians. In this study, none of the samples yielded *Mycoplasma*, and it is believed, therefore, that the organisms are not normal udder microorganisms, but rather that they reside elsewhere and, when present in the udder, result in obviously severe clinical disease.
Studies of four experimental cows showed that severe incurable mastitis developed promptly in cows whose udders were infused in one quarter with an inoculum of *Mycoplasma* culture that contained approximately 100 organisms, by colony count. The infection spread to other quarters within a few days period. The adjacent gland on the same side as the infused gland was always involved first. Our cows were machine milked. Similar experience in machine-milked cows has been reported by Hale, et al., and Stuart, et al. In Stuart's experience, Strain "W" did not spread readily from infected to normal quarters in cows milked by hand. Of particular significance in our herds was the rapid drop in production and the failure of infected cows to recover and return to production. One experimental cow continued to shed organisms in the mammary secretions from all quarters (which appeared similar to cottage cheese in a serum menstruum) throughout the lactation and dry periods. This animal calved normally and the calf was fed approximately two quarts of frank pus, which represented thecolostrum. The calf failed to show signs of illness and developed normally, as did other calves fed infective mammary secretion. The cow was slaughtered four weeks after calving, six months after the initial exposure to *Mycoplasma*, and organisms were recovered from all quarter secretions and tissues and also from the supramammary lymph nodes.

To determine possible methods of spread of infection, abnormal secretion was obtained from the udder of an experimentally inoculated cow that had been infected for approximately five weeks. The right rear and right fore teats of a second machine-milked cow were exposed by simply dipping them to a depth of one cm. for 10 seconds in the secretion. This cow was producing almost 60 pounds daily when exposed. Following this single exposure, the cow showed severe purulent mastitis in the exposed quarters within 36 hours and by the ninth day after exposure, all four quarters were swollen and firm. Production dropped to 17 pounds within 48 hours and after two weeks the cow was essentially dry, with the exception of a small amount of purulent, caseous secretion.

Our present experience, wanting conclusive proof, leads us strongly to suspect that *Mycoplasma* infected udders and contaminated syringes and test cannulas were responsible for the outbreaks that occurred. These infections resulted in seriously damaged udders in a high proportion of the cows infected. In fact, many of the cows positively diagnosed by *Mycoplasma* isolation had been removed from the herds before second samples could be procured one month later. Marked atrophy of the quarters appeared following the acute attack in the experimental cows and also in some cows naturally infected. This resulted in the eventual sale of cows not sold at once because of firm edematous udders and the total absence of profitable production.

Some cows in a few herds developed the disease without having been infused with antibiotics; also many were not in stanchions adjacent to cows known to be infected. This suggests the possibility of an additional source of infection other than the teat orifice. One experimental cow developed a deep cough 19 days after *Mycoplasma* had been infused into one quarter
and identical Mycoplasma were isolated from the nasopharynx. Mycoplasma showing characteristics of Strain "F" also have been isolated from the nasal cavities of a few cows in herds where the organisms were proved the cause of mastitis. Such cows did not show respiratory symptoms, nor did these cows show mastitis either at the time of sampling, or later. The possibility that an aerosol infection from the respiratory tract in crowded stables may be a factor in the spread of Mycoplasma mastitis cannot, therefore, be ignored. Obviously, much more study is needed to determine the epizootiology of this infection.

**DIAGNOSIS**

A positive diagnosis can be made only by culture of the milk on media that will support the growth of Mycoplasma. The media outlined herein have proved satisfactory, although others\(^3\) have employed simply horse blood broth or blood agar incubated for 48 hours in an atmosphere of 10 percent carbon dioxide. Because infected glands may contain other microorganisms, in addition, it would be necessary to add bacterial inhibitors to blood agar plates. The finding of characteristic colonies (Figures 1 and 2) is sufficient for diagnostic purposes, pending additional tests to characterize the agent, e.g., hemolysis of horse blood agar.

A tentative clinical diagnosis is possible in some herd outbreaks from the history and signs mentioned; nevertheless, we have found that from clinical signs alone a positive diagnosis is impossible. Laboratory tests are essential in order to establish with certainty the nature of the infection, especially since natural infections frequently are complicated by common bacterial infections appearing concurrently. There was no consistent concurrent bacterial infection; however, *Streptococcus agalactiae*, non-agalactiae streptococci, hemolytic and non-hemolytic staphylococci, *Corynebacterium pyogenes*, coliform bacteria, and bacilli were identified in milk samples yielding Mycoplasma.\(^{10}\)

The single fact that many cows in a herd may have a mastitis that does not respond to any common method of treatment and whose udder secretions are negative on usual cultural media (bovine blood agar), should lead to an examination for Mycoplasma. This would be especially true in those cases where treatment has not been given for several days. Affected udders shedding Mycoplasma usually feel hard enough to be classified as fibrotic, although the very hard, usually painless, smooth swelling is not due to pure fibrosis, but rather to granulomatous changes occurring within the udder. The absence of signs of toxemia, as seen in coliform and other forms of acute mastitis, is usual but, as noted, experimentally infected cows showed an early rise in temperature. The udders more closely resemble the great hypertrophy seen in mycobacterial mastitis, described by Tucker.\(^{11}\) In *Mycoplasma* mastitis, neither acid-fast organisms nor "giant-cells" were found, and the character of the secretion was usually a yellow serum with chunks of coagulated material, rather than
watery milk and flakes or pus as found in mycobacterial infection. Both types of mastitis have one sign in common, however, in that oily vehicles for infused antibiotics appear to stimulate production of huge, heavily swollen udders.

The prognosis is not good for an individual cow, but herds may resume normal production after culling of cows affected with this form of mastitis.

**PREVENTION AND TREATMENT**

A complete plan of good management normally used by dairymen co-operating with the NYSMCP appears to reduce chances of experiencing *Mycoplasma* mastitis, although data are lacking on the mode of infection. The program should include careful isolation of all purchased cattle that are old enough to be pregnant, and it seems especially important to avoid purchase of adult cattle from public sales unless they can be housed and milked separately for several weeks. Our data indicate that several of the outbreaks occurred after the introduction of cows purchased at private or public sale. Many of these cows had been sold because of mastitis before they could be examined; thus our current impressions are based partially upon presumptive evidence.

Veterinarians and herdsmen should use strict asepsis, or at least surgical cleanliness, when performing all udder infusions. Especially important is the avoidance of syringes with rubber plungers that cannot be adequately or easily sterilized when moving from one herd to another, or even from one cow to another in the same herd. The use of individual syringes and cannulas, with disinfection of the operative hands between cows always is desirable, especially when confronted by a contagious infectious agent capable of causing incurable disease.

None of the common antibiotics available for mastitis therapy which showed *in vitro* inhibition have proved effective in natural or experimental cases. The observation was made repeatedly that the usual antibiotics suspended in oil-in-water emulsion when infused into the udder increased the severity of *Mycoplasma* mastitis. Daily intravenous injections of five grams of oxytetracycline hydrochloride (Liquamycin), to which the organisms show *in vitro* sensitivity, for three consecutive days showed slight beneficial effect because the udder secretion increased from a few ounces to a liter per day and the udder became slightly less firm. This antibiotic halted recovery of organisms from the gland secretions during the period of treatment and for two days after it was withdrawn. Nevertheless, recrudescence of edema and purulent secretion occurred within two weeks. Repeated stripping out of the white tapioca-like masses of odorless material from infected quarters during the dry periods when the udder was undergoing involution may have helped one experimental animal, which produced a fair amount of normal milk at her next lactation after a long rest for the mammary tissue.
BOVINE MYCOPLASMA MASTITIS

Control, in view of our present lack of better information, is apparently dependent upon immediate slaughter of all clinical cases, or at least complete isolation of such animals until their udders are normal and the udder secretion is proven free from Mycoplasma. Because these organisms have been shown experimentally to persist for at least six months, the hazards of a potential source of infection in a problem herd

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>Economic Losses From PPLO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td><strong>Average</strong></td>
</tr>
<tr>
<td><strong>PPLO Herds</strong></td>
<td><strong>Size</strong></td>
</tr>
<tr>
<td>N.Y.S.</td>
<td>Herd</td>
</tr>
<tr>
<td>15</td>
<td>40 cows</td>
</tr>
</tbody>
</table>

An average of 33.1 percent of cows were sold on account of mastitis from 13 Mycoplasma herds.

(High 77 percent - Low .04 percent)

probably do not warrant maintenance of an animal unless for exceptional reasons.

ECONOMIC IMPORTANCE

In Connecticut, Hale\(^3\) reported serious damage to one herd and later\(^9\) reported that isolations had been made from five or six additional herds during the last two years. In England,\(^2\) the Mycoplasma caused greatly reduced milk yield for several weeks followed by clinical recovery within one to four weeks, after which these animals did not appear to have suffered any permanent udder damage. They gave satisfactory milk yields in subsequent lactations. Similar experience was obtained in our studies\(^7\) with Strain "56R". But in contrast, cows infected with Mycoplasma identical with Strain "F" (and the Connecticut Strain "D"), showed severe residual udder damage and, thus far, we have been unable to detect antibodies in the convalescent animals or whey by either complement-fixation or agglutination tests. Complement-fixing antibody readily was detected following recovery from infection by Strains "56R" (New York) and "W" (England). Our search for Mycoplasma mastitis has shown 13 seriously damaged herds in New York State during the last nine months.\(^10\) Isolations were made from two herds that suffered apparently little damage, with only one or two cows affected that promptly were slaughtered.

Fourteen herds, containing 644 cows, were studied after the cause of severe mastitis in these herds was discovered to be Mycoplasma. Data is lacking on the number of cows in one other herd affected with Mycoplasma mastitis. In the herds where reliable data were available, 197
cows (33.1 percent) were sold because of apparently hopeless incurable mastitis from 13 of the herds that had 594 cows. Abnormal secretion was observed in seven herds at the time the initial outbreaks occurred, and this averaged 49.4 percent of the 283 cows in these herds. This is 32.9 percent greater than the 16.5 percent abnormal secretion rate found in 395 new herds that entered the NYSMCP in 1962. The udder tissue also was much more seriously damaged in the Mycoplasma infected cows than in the other 395 herds entering the program for the first time.

We feel, therefore, that Mycoplasma infection is a serious threat to the health of the udders of cows in herds that develop this infection. Although the number of affected herds does not seem large at present, the isolation rate seems to be increasing as we become more aware of the problem and alert veterinarians to characteristics of the disease, as now recognized. Frequently, only one or two cows were involved, and the trouble disappeared after these were sent to slaughter (or sale).

SUMMARY

A clinical and laboratory study was made of cows in 15 New York State herds with mastitis caused by Mycoplasma. Natural and experimental infections by these organisms caused serious damage that generally resulted in the removal of infected animals from affected herds.

Methods are described for isolation and recognition of the causal organism, and clinical information is presented to help enable veterinarians and regulatory officials to recognize and restrict the spread of this recently recognized disease of the bovine mammary gland.

A study of more than 500 milk samples from 27 herds in six counties cooperating with the New York State Mastitis Control Program failed to reveal Mycoplasma. Mycoplasma have not been found in milk that is normal.

Slaughter of cows with infected and severely damaged udders enabled affected herds to resume normal production.

ACKNOWLEDGEMENT

The authors wish to acknowledge with appreciation the assistance of the following practitioners who cooperated in this study: F. C. Cairns, R. E. Lormore and E. D. Mackey.

REFERENCES

REPORT OF THE COMMITTEE ON MASTITIS

J. F. Quinn, Lansing, Michigan, Chairman; H. S. Bryan, Kalamazoo, Michigan; J. H. Drayer, Columbus, Ohio; R. W. Metzger, Syracuse, New York; G. W. Reed, East Lansing, Michigan; R. J. Schroeder, South Gate, California; Dr. Harry Hodges, Phoenix, Arizona

It has been recognized for many years that Mastitis is the most costly of all livestock diseases from an economic standpoint. Contrary to other diseases and their control therein, Mastitis cannot be attacked by enacting regulatory measures as to the movement and maintenance of livestock.

The dairy cattle owner and his attending veterinarian must be educated as to the proper herd maintenance practices for the prevention of this disease, as well as the accepted techniques for its detection and treatment when found to exist in a herd. This education procedure, or lack thereof, has long been a stumbling block to successful Mastitis control programs.

The Mastitis Committee, having met during the 67th meeting of the United States Livestock Sanitary Association, would like to present the following resolutions.

1. The promotion of so called cure-alls for mastitis has interfered with Mastitis Control.

2. Whereas, contrary to its tremendous economic and public health impact, little or no public funds have been appropriated to assist control programs.

3. Whereas, Laboratory facilities using accepted uniform techniques for detecting this disease have not been available. States should be urged to provide these laboratories to the industry and the Veterinary Profession.

4. Whereas, Mastitis is not essentially a regulatory responsibility. However its control can be greatly enhanced by close collaboration between disease control agencies and the National Mastitis Council who has accepted the responsibility of setting forth principles of a sound control program.

Therefore it is urged that the Executive Committee direct that continued close collaboration be maintained between the United States Livestock Sanitary Association and National Mastitis Committee and that the former give serious consideration to financial assistance where it is needed.
REPORT OF THE COMMITTEE ON PARASITIC DISEASES

F. R. Koutz, Columbus, Ohio, Chairman; J. L. Hourrigan, Arlington, Virginia; D. W. Baker, Ithaca, New York; F. D. Barrows, Cheyenne, Wyoming; R. L. Cuff, Kansas City, Missouri; F. D. Enzie, Beltsville, Maryland; L. E. Swanson, Gainesville, Florida; W. C. Tobin, Denver, Colorado; F. B. Wheller, Baton Rouge, Louisiana

In the early days of its inception, this Association devoted a major portion of its time and efforts to parasitic diseases—namely, cattle fever ticks, sheep scabies, and cattle scabies.

Your Committee thought it appropriate to briefly mention progress made in these areas.

CATTLE FEVER TICK ERADICATION

An all-out tick eradication program was instituted in 1906 following the far-reaching research discovery that *Boophilus* ticks served as vectors of piroplasmosis. 37 years later, in 1943, the tick had been eradicated from the United States, except for a narrow buffer zone, under Federal and State quarantines along the Texas-Mexico border. These, reinfestations occur from time to time and an active program is required to prevent additional spread into adjacent areas. Reinfestations have also occurred in California and in Florida.

The eradication program includes inspection, quarantine, and dipping of infested animals.

Prevention—keeping the ticks out of the United States—is now the major part of the effort against cattle fever ticks. The quarantine zone is maintained along the international boundary and the lower Rio Grande River in eight Texas counties as adjacent areas in Mexico are infested. Cattle from Mexico are carefully inspected for ticks at the border. They must be free of ticks and must be given a precautionary dipping before they can be imported.

Without these controls, cattle fever ticks would reinfest areas of the United States that have warm climates. In spite of continued efforts to keep out these parasites, they have reappeared from time to time, but vigilance and prompt eradication measures have eliminated the outbreaks.

Should the tick gain a foothold, piroplasma-carrier cattle imported from Mexico could furnish reservoirs leading to heavy losses in our cattle population. These ticks also serve as vectors of other diseases.

Active Program Continued in Texas

During fiscal year 1963, in the buffer area in Texas, 313 livestock illegally crossing the border were caught; 39 tick-infested United States herds were found; and 65,775 lots of 1,726,009 livestock were inspected for ticks and 12,453 lots of 91,333 livestock were dipped.
Special Tick Survey Along Rio Grande River

In July and September 1962, live cattle fever ticks were collected from two stray Charolais bulls found approximately four miles north of the area under State and Federal tick quarantine. An intensive survey made by four inspectors during April, May, and June did not reveal any additional infestation. The Amistad dam, scheduled for completion in 1967, will form a reservoir extending some 80 miles above Del Rio, Texas, and should then aid materially in preventing animals from straying from Mexico into the area concerned.

Boophilus Ticks Found Outside Quarantine Area

During routine inspection of a calf at an auction market in Gregg County, Texas, two dead immature ticks were collected. The ticks were subsequently identified in Texas and at Beltsville, Maryland, as Boophilus. Epidemiological studies traced the shipment, of which the calf had been a part, back to Louisiana where 1,930 cattle and 18 horses belonging to 20 owners were inspected. No additional Boophilus ticks were found.

Inspection Facility and Cattle Dipping Vat Plans Distributed

A three-page plan showing details of recommended cattle inspection facilities and a dipping vat was printed and distributed in September 1962. The prints will also be available in each State through the Cooperative Extension Service. These plans are a composite of a large number of plans reviewed and include the best features of all.

Parasite Identification at USDA Parasite Reference Center

The National tick survey continued and during fiscal year 1963, tick specimens totaling 2,383 were received and identified at the Parasite Reference Center, Beltsville, Maryland. Also, 94 mite specimens and 71 miscellaneous parasite specimens were identified. Of approximately 31,459 lots of suspected screwworm larvae received, some 27,314 were identified as screwworm and 4,459 as various species of blow fly larvae.

Additional Information Concerning Cattle Fever Ticks (Boophilus microplus) Developed in the Virgin Islands

Dr. Robert L. Park, USDA Animal Husbandman, developed information that larval ticks obtained as a result of incubating eggs from engorged female ticks removed from cattle would develop fully on deer, and that the eggs from such engorged female ticks maturing on deer would hatch.
The Eradication Program

A review of previous proceedings of annual meetings of this Association clearly indicates that your emphasis placed upon the urgent need for complete eradication of psoroptic sheep scabies has "borne fruit."

In 1954, the United States Livestock Sanitary 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 announced that a tentative plan for the eradication of the disease had been developed. Largely through the efforts of this organization, the plan was accepted with certain modifications by all concerned and put into effect by amending appropriate Federal regulations. Thus, Part 74, Title 9, CFR, was amended effective August 1, 1960. The amended regulation designated free and infected States and provided for the designation of eradication areas when State and Federal officials mutually agree to a comprehensive eradication program as outlined in ADE Division Memorandum No. 505.6, dated November 21, 1960. The new regulations also gave better protection to the free areas.

The movement of sheep not known to be infected or exposed between States in the infected area was not controlled by the regulation until May 1963 when an amendment became effective requiring the dipping of such sheep unless consigned to a public stockyards or a recognized slaughtering center.

Excellent progress has been made toward eradication, since the accelerated sheep scabies eradication program began in August 1960. At that time, the only area actively engaged in an eradication program comprised 44 counties in eastern South Dakota. There were 1,421 counties considered Sheep Scabies Free and the remaining 1,689 counties were classed as infected. At present, there are 2,420 counties considered Free, 420 counties actively engaged in eradication and only 314 counties in four States that have not yet qualified as Eradication Areas.

Regulatory officials have long recognized that when a disease reaches a low incidence level and is not pursued relentlessly to complete eradication, complacency often develops. An increase in the incidence of the disease usually follows, eliminating previous progress. This had occurred in the Midwestern and Middle Atlantic States and the incidence reached a high level. Sheep scabies had continued to be prevalent in spite of individual eradication efforts of several States from time to time. These States have been sources of infection to Scabies Free States. The all-out endeavor to bring about complete eradication of sheep scabies began using roll-back procedures with major efforts concentrated at the periphery of the infected areas to decrease the size of the infected area and to give greater protection to Free Areas. Considerably more effort will be
required to get the job done in the remaining infected areas where larger numbers of farm flocks and a higher incidence of the disease exists.

It is apparent that the opportunity exists to push sheep scabies eradication to completion within a relatively short period of time if the present degree of national enthusiasm can be maintained. However, complete eradication will depend largely upon increased cooperative efforts of all those interested in the health aspects of our nation's sheep population and in the continued economy of the sheep industry.

Inspection and Dipping Activities

Psoroptic scabies was reported in 268 flocks of 20,160 sheep in 180 counties in 21 States during fiscal year 1963, compared to the report of 767 infected flocks in 316 counties in 24 States the previous year. 51 infected lots were found at public stockyards compared to 121 during 1962. 15,500,000 sheep were inspected during 1963 and 843,000 dipped—a considerable increase over 1962 when 12,770,000 were inspected and 590,000 dipped.

Outbreaks in the Sheep Scabies Free Area

Mississippi reported four infected flocks; Kansas, North Carolina and Wisconsin each reported two infected flocks; and Colorado, Delaware, and Nebraska each reported one infected flock during fiscal year 1963.

15 Entire States and Territories and Parts of Four Other States Succeed in Eradicating Sheep Scabies

The following States and territories, declared infected or partially infected in August 1960, are now Sheep Scabies Free: Alaska, Arkansas, Kansas, Maryland, Michigan, Minnesota, New Jersey, New York, North Dakota, Oklahoma, Pennsylvania, South Dakota, Wisconsin, Virginia, and the Virgin Islands. Parts of Hawaii, Illinois, Nebraska, and Missouri have also been freed of the disease.

New Concept of Rapid Sheep Scabies Eradication Adopted by More States

This concept, discussed for many years by members of our Association has been put to the test in Wisconsin, Maryland, Minnesota, Oklahoma, and Virginia. We are encouraged by the apparent good results and are watching closely to determine whether this method will stand the test of time.

CATTLE SCABIES ERADICATION

During fiscal year 1963, nearly 13 1/2 millions of cattle were inspected for this disease—a decided increase over the previous year. For the first time since 1952, psoroptic cattle scabies was not reported in the United States. The most recent series of outbreaks had their beginning in 1954 when 28 infected herds were found in 15 counties in six States. Strenuous efforts to develop the epidemiology of each outbreak and to locate all infected and exposed animals were made during the intervening
nine years. There was a gradual reduction in the number of outbreaks; however, psoroptic mites were found on cattle in many areas involving the States of Arizona, California, Colorado, Illinois, Indiana, Iowa, Kansas, Kentucky, Missouri, Nebraska, New Mexico, Ohio, Oklahoma, Texas, Washington, Wisconsin, and Wyoming. The earlier outbreaks were found largely in range cattle. More recently, the disease was found more often in feedlots, particularly those receiving cattle from the range areas found previously to be involved. Several of the outbreaks were disclosed when infected cattle reached public stockyards. Other outbreaks were found through the efforts of veterinary practitioners and through "down-the-road" inspections of large numbers of cattle in the range areas concerned, as well as through exhaustive tracing of the movements of cattle both to and from herds in which outbreaks occurred. Areas where intensive inspections were made included particularly the general area involving parts of Colorado, Kansas, Oklahoma, Texas, and New Mexico.

It is, of course, not possible to predict what may happen in the coming years insofar as outbreaks are concerned; however, efforts to determine if additional reservoirs of the disease may exist are continuing.

Available reports of psoroptic cattle scabies go back some sixty years in which the disease appeared quite regularly in the majority of the western States and eradication programs were begun. Cattle scabies was brought under control and the incidence of the disease decreased considerably; however, reinfections were not uncommon and during the period 1920 to 1929, active inspection and dipping programs were carried out in Arizona, Colorado, Kansas, Montana, Nebraska, New Mexico, Oklahoma, South Dakota, Texas, Utah, and Wyoming where outbreaks were frequently found and in other areas where the disease was less prevalent. These efforts were eventually successful; however, it was not until 1950 that cattle scabies was not reported in the United States. No reports were received in 1951 or 1952 and thus the series of outbreaks which began in 1954 were the first for several years.

STATUS OF SCREWWM ERADICATION PROGRAM

The southwestern screwworm eradication program has successfully reduced screwworm populations in the eradication area to an extremely low number. Although screwworm numbers have been limited compared to last year, the area of infestation has been comparatively great due to the fly migrating distances much greater than previously thought and to animal movements.

Due to the difference in climate and topography of the southwestern States from the southeast, modifications and improvements have been made in the techniques of caring for the sterile flies and the aerial release of the sterile flies over the most favorable habitats. As many as 140 million sterile flies per week have been dropped. No screwworms have been found this year in the southeastern States or in Arkansas and Louisiana.

A sterile fly barrier zone has been established along the United States-Mexico international border to aid in preventing the entrance of
screwworm flies from Mexico. The effectiveness of this barrier is being evaluated.

The inspection line along the eastern borders of Arkansas and Louisiana continues to be operated and nearly 1/2 million animals entering the free area in the southeast have been unloaded, inspected, and treated with an appropriate pesticide. An additional inspection line has been established along the western border of New Mexico to prevent screwworms from entering the southwestern eradication area from States further west.

RECOMMENDATIONS OF COMMITTEE ON PARASITIC DISEASES

Your Committee noted that exotic ticks again found their way into the United States—this time, in the ear of an imported rhinoceros.

We wish to call the attention of the Association to our last year's resolution that importation requirements be amended so as to preclude further introduction of exotic external parasites. Our resolution outlined specific recommendations and appears in the published proceedings of last year.

Your Committee notes that for the first time since 1952, no psoroptic cattle scabies was reported. The States concerned are to be commended for their efforts and for increasing the number of cattle inspected to a high of nearly 13-1/2 million. We recommend that the exhaustive efforts to completely eradicate the disease be continued.

In further regard to cattle, we note that psorergatic mites were reported for the first time, according to the literature, on this species. The one infested animal is the subject of research studies at the Animal Disease and Parasite Research Division Laboratory in Albuquerque.

We recognize that as successful programs reduce the incidence of certain external parasites, additional efforts must be devoted to improving program procedures and techniques.

We, therefore, recommend that all responsible Livestock Sanitary officials devote additional time and effort in the areas of vat management, uses of pesticides, training of personnel, and development of capability in each State for conducting the maceration technique for scabies diagnosis.

In also recognizing the vastly increased attention pesticides are receiving, and their increasing importance, we recommend that the name of this Committee be broadened to be:

COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES.

This is in keeping with the functions this committee has actually been performing for many years.

The considerable progress made in the Sheep Scabies Eradication Program was reviewed and the Parasitic Diseases Committee is pleased to announce that 15 entire States and territories and parts of four (4) others have succeeded in eradicating this disease since August 1960.

Be it resolved that the United States Livestock Sanitary Association urge that the Secretary of Agriculture request, and that the Congress appropriate adequate funds in the amount of $1,450,000.00 to join with the
various States for the final drive to eradicate psoroptic sheep and cattle scabies from the United States.

Your Committee received a resolution from the North Central Livestock Sanitary Officials Association that minimum and uniform standards be established for areas which have achieved a scabies-free status. We believe this is a timely resolution and the committee will draft suggested standards for the purpose of review.
POULTRY HEALTH CONTRIBUTIONS BY PRIMARY BREEDERS

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The rapid growth and development of the broiler industry during the last fifteen (15) years was made possible by major advances in feed, management and genetics. Among these advances were high energy feed, automation, and the increase of flock sizes in concentrated geographic areas. This latter practice made it inevitable that the research responsibilities of the primary breeders must now include investigation of the prevention of embryo passed diseases as well as the previously accepted genetic responsibilities. The fact that a very high percent of the total broiler breeders placed are produced by a very small number of primary breeders further accentuates research responsibility for their health.

The most costly disease of the broiler industry during recent years has been chronic respiratory disease. Professional researchers from colleges, government and the industry cooperated with the United States Department of Agriculture to find a satisfactory method to control or prevent the disease. From these published sources of information and through our own research and observations, it appeared that probably CRD could be eradicated.

By 1956, we had established our first CRD free flocks. During the next six (6) years, our research and development division studied and observed the disease and practical applications of its eradication. Early experiments proved embryo transmission to be variable; and at specific periods that could be predetermined, it was very low. The disease could spread very rapidly from a few infected chicks to the entire flock; but the organism is very fastidious and lives for only a very short period of time outside of the host.

This long experience in the practical application of CRD eradication proved to us that it could be accomplished by the entire broiler industry. By 1962, we had expanded the program to a majority of our commercial breeder lines and introduced CRD eradication to limited numbers of selected customer accounts in each of the major broiler areas of this country and Canada.

Now, more than a year after initial commercial introduction, field results are proving practicability of the program. More than a million and a half broiler breeders are growing on typical poultry farms throughout the country. To date, more than 90 percent of those flocks reaching maturity and their broiler progeny have remained free of CRD. Growth rate and feed conversion have been improved, condemnation for air sac infection has been drastically reduced; and all this has been accomplished without the high cost of medication for CRD.

Other primary breeders have been the advantages and commercial acceptance of CRD eradication; and it now appears quite probable that this
POULTRY HEALTH CONTRIBUTIONS

disease will, within the next five years, be reduced to minor economic im-
portance in the broiler industry.

Primary breeder responsibility to investigate methods of disease pre-
vention does not end with eradication of CRD. For many years, leukemia
has been responsible for the highest economic loss due to disease in the
poultry industry as a whole. During recent years, a specific form or strain
of this disease with acute epidemic manifestations in comparatively young
birds, usually seven to 17 weeks of age, has evidenced rapid spread in the
broiler phase of the industry. Replacement flocks have been affected most;
but condemnation due to lesions of leukemia in broiler flocks is increasing
at an alarming rate. It appears to resemble Marek's disease first re-
ported in Austria in 1907.

While a few research stations have for a long period of time special-
ized in work with avian leukemia, a really concentrated approach by large
numbers of researchers in separate institutions is only beginning. With
leukemia, as with CRD, technical research must show the way, and the pri-
mary breeders must apply these research findings to practical application,
be it eradication, immunization, genetics or treatment by medication.

Of particular interest with leukemia is the high susceptibility of young
chicks as compared to increasing resistance with age. The disease ap-
ppears to linger on farms between batches of chicks sufficiently to promote
exposure to following broods and evidence has shown that it may be air
transmitted to reasonable distances under specific circumstances. But
the agent is very susceptible to certain disinfectants and fumigants; and
while this may require changes in brooding facilities to eliminate dirt
floors to make houses susceptible to sanitation, it does suggest the be-
inning of use of research information to reduce economic loss due to leu-
kosis.

The industry, by virtue of its economic losses, tends to bring atten-
tion to one major disease at a time; but as breeders have long accepted
the responsibility for selling pullorum free chicks, so do they constantly
guard against infection of many other diseases in their flocks. Among a
long list of these are various members of the Salmonella group, cholera,
AE, and several respiratory diseases. And recent research progress in
developing a test for infectious synovitis places another disease control
tool in the hands of the primary breeder.

However, the industry all too often demands (and all too often gets)
indemnity by adjustments for disease losses over which the breeder has
no practical control. Galloping leukemia (or Marek's disease) is, at the
present time, a good example; because until research has shown the way,
and the breeders have been able to make practical application of a con-
trolled program, there is no way by which they may control such a disease
on the farms of their customers. No breeder should be expected to adjust
for losses for a disease they have no way of controlling and then be ex-
pected to pour money into research on that disease at the same time.

Some poultry diseases, like pullorum and typhoid, have been satis-
factorily controlled by industry-requested testing programs on a national
scale. It would appear that practical eradication of CRD in the broiler
segment of the industry could be attained in a similar or joint program.
Some areas or states may be able to prevent contamination of flocks from specific diseases by geographic or quarantine barriers. Those poultry diseases which are pathogenic to humans are reportable and fall under strict control regulations.

You gentlemen who set the rules for your states are undoubtedly often faced with a choice of quarantining diseases and genetically desirable stock at the same time. And more often than not, your technical decisions may not be popular with the commercial industry. But your decision may often determine the future prosperity of the industry in your state.

Certainly, now that industry leadership has been reduced to fewer and fewer individuals, so has the opportunity increased for closer cooperation in the area of poultry health. Primary breeders can best help themselves and improve the acceptance of their products by supporting practical poultry health regulations and you will find us most cooperative. But more than that, industry control of embryo-passed diseases must originate with the primary breeder; and we must look to the poultry researchers for the necessary technical advice to find the way; thus we all may serve our industry best by cooperating in determining the most effective and practical control measures and the most effective time to put such controls into effect.
FOWL CHOLERA: SUSCEPTIBILITY OF VARIOUS ANIMALS AND THEIR POTENTIAL AS DISSEMINATORS OF THE DISEASE

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The fowl cholera organism, Pasteurella multocida, usually enters the tissues of poultry through mucous membranes of the pharynx or upper air passages. In acute fowl cholera there is an overwhelming hemal multiplication usually resulting in death. Some birds recover and still carry the organism in their nasal passages. Pasteurella multocida was isolated periodically over a four-year period from the nasal pharynx and eyes of chickens that survived an acute outbreak. The source of infection in a flock may be traced to birds that have survived an outbreak but are still maintained on the premises as breeding stock. However, outbreaks also occur with no indication as to the source of infection. Poultry and free flying birds are easily infected by subcutaneous and intramuscular inoculation. They can usually be infected by instillation of culture in the conjunctival sac, or on the nasal or laryngeal mucous membrane. Horses, cattle, sheep, pigs, dogs and cats are refractory to infection per os. However, all of these animals may succumb to intravenous inoculation of moderately heavy doses.

A comparison of the susceptibility of various species of animals to the same avian strain of P. multocida, administered via the respiratory tract, could not be found in the literature.

The purpose of this experiment was to determine the susceptibility of certain domestic farm animals, rodents and free flying birds to a highly virulent strain of fowl cholera organism and their potential as a reservoir of infection.

EXPERIMENTAL METHODS AND RESULTS

Culture of Pasteurella multocida. The strain used was a lyophilized culture of X-73. The culture was resuspended in one percent peptone broth and streaked on Dextrose Starch Agar. A suspension was made of the colonies in one percent peptone broth to a McFarland number one density (approximately $1.34 \times 10^9$ bacteria per ml.), which was the concentration for exposure. The virulence was such that when the culture was


The authors are grateful to Dr. K. R. Rhoades of the National Animal Disease Laboratory for the histopathologic report.

**Difco Laboratories, Inc., Detroit, Mich.
diluted to $10^{-4}$ and 0.1 ml. was injected intramuscularly (IM) in each of five chickens, five rabbits and five mice, all animals died within 24 hours.

**Animals**

Before exposure, the nasal pharynx or nostril of each animal was swabbed with a cotton-tipped, six-inch wooden applicator. The swabs were twirled in a tube containing 10 ml. of a one percent peptone broth (pH 7.3 to 7.4). The broth samples were incubated at 37°C for two to three hours and streaked on Dextrose Starch Agar* and beef infusion agar containing five percent defibrinated bovine blood. After incubation at 37°C for 24 hours, the plates were examined and found to be negative for Pasteurella.

**Pigeons.** Two groups, 12 and five, of common pigeons (*Columbia livia*) taken from a barn loft were given 0.05 ml. of culture intranasally (IN) and one part culture in four parts drinking water respectively.

The 12 pigeons exposed IN died of acute fowl cholera. Three of the 12 pigeons died in one day, four in two days, three in three days, and two in four days. Three of five pigeons given the culture in drinking water died in 24 hours. The remaining two pigeons were killed six days after exposure and *P. multocida* was isolated from the palatine cleft and abdominal cavity of one of the pigeons.

**Sparrows.** Twenty English sparrows (*Passer domesticus*) taken from various farm buildings were divided into two equal groups; the first group was given 0.05 ml. of culture IN, and the second group was given one part culture in four parts drinking water.

All of the sparrows died of acute fowl cholera in 24 hours. At necropsy, *P. multocida* was isolated from the breast muscle and liver of all birds.

**Rats.** Seventeen young white rats, (96 grams average weight) were exposed to the culture. Two groups, five and two, of rats were given intraperitoneal (IP) injections of 0.1 ml. and 0.2 ml. respectively. Five rats were inoculated IN with 0.1 ml. of culture, and five rats were fed the viscera of two chickens that died of fowl cholera. A piece of liver from each chicken, when streaked on agar plates, produced a solid growth of *P. multocida*.

One rat given 0.2 ml. of culture IP died in 48 hours. *Pasteurella multocida* was isolated from heart blood and liver. One rat fed infected viscera developed nasal congestion with an accumulation of pus from which *P. multocida* was isolated 11 days after exposure. There was no observable evidence of infection in the remaining rats.

**Mice.** Twenty-six Swiss-Webster mice, 20 grams average weight, were exposed to the culture. Seven mice were inoculated IN with 0.05 ml. of culture. Nineteen mice were fed the viscera from four chickens that died of acute fowl cholera.

*DIFFCO Laboratories, Inc., Detroit, Mich.*
Five of seven and 11 of 19 mice given an IN exposure and fed infected viscera respectively, died in four days, with the majority dying in two days. *Pasteurella multocida* was isolated from the heart blood and livers of all mice that died.

**Rabbits.** Five White New Zealand young mature does were inoculated IN with 0.05 ml. of culture.

In all rabbits there was lacrimation, nasal discharge, gasping respiration, cyanosis and death within 24 hours. *Pasteurella multocida* was isolated from the heart blood and livers of all rabbits.

**Guinea pigs.** Fifteen Hartley strain guinea pigs, (347 grams average weight) were exposed to the culture. Five guinea pigs were inoculated IN, five IM, and five IP with 0.1 ml. of culture each.

Intranasal exposure failed to elicit any noticeable response. In the guinea pigs given IM injections, there was loss of weight and hair, and necrosis at the site of injection. Three of five guinea pigs given IP injections died in 24 hours and *P. multocida* was isolated from the heart blood and livers.

**Mink.** Six mink from different litters were obtained from a commercial mink ranch. Two 24-week-old mink were fed for five days the viscera of three chickens that died of acute fowl cholera. One seven-week-old and three seven-week-old mink were given 0.1 ml. of culture IP and 0.2 ml. IN respectively.

One of the 24-week-old mink had a rectal temperature of 106°F and the other had 107.6°F on the fourth day, the only day their temperatures were taken. Both mink had a greenish diarrhea for five days. When killed 12 days after exposure, one mink had pneumonia and *P. multocida* was isolated from the lung.

The seven-week-old mink that was given an IP injection died in eight hours. Immediately before death the respiration was 40 and the rectal temperature 100.4°F. *Pasteurella multocida* was isolated from the heart, liver, kidney, spleen and peritoneal cavity.

There was anorexia, and a rectal temperature of 107°F for two days in one of the mink exposed IN. On the third day the mink was eating and the temperature was 100°F. The animal was killed on the sixth day and at necropsy no gross lesions were observed and no *P. multocida* was isolated. The rectal temperatures in the remaining two mink were between 99°F and 100°F and there were no signs or lesions of infection.

**Ferrets.** Four four-month-old ferrets from the same litter were exposed to the culture. Two ferrets were inoculated IN with 0.2 ml. of culture, and two ferrets were fed for five days the viscera of chickens that died of fowl cholera.

There was no noticeable response in any of the ferrets. At necropsy there were no gross lesions, and *P. multocida* was not isolated.

**Calves, sheep and pig.** Two male 15-week-old Holstein-Friesian calves, one 43-week-old crossbred sheep (Western ewe and Suffolk ram), and one 15-week-old male York pig were exposed to the culture. One calf
was given six ml. IM in the neck, and the other calf, a sheep and a pig were given two ml. in each nostril and per os. Thirty-four days later, the calf, sheep and pig that were exposed IN and per os were each injected with three ml. IM in the neck.

The calf given six ml. IM developed septicemia and died within 18 hours. At necropsy there was a generalized passive congestion, hemorrhages, edema, and early coagulative necrosis of muscle fibers.

There was no observable signs of disease in the calf, sheep, or pig exposed IN and per os. Pasteurella multocida was isolated from the nose of the calf and pig 34 days after inoculation. After the three animals were given a second exposure by the intramuscular route, there was anorexia for 24 to 30 hours; rectal temperatures rose within six hours from 101.8°C to 104.4°C, from 102.6°C to 107.0°C, from 102.7°C to 106.1°C, and remained above 103.0°C for seven, seven and three days respectively in the calf, sheep and pig.

Three groups of ten 12-week-old New Hampshire chickens were exposed to the culture that was re-isolated from the nose of the pig. The first group was given 0.1 ml. IN, the second group one part culture mixed with two parts feed, and the third group one part culture mixed with four parts drinking water. Of the chickens exposed to P. multocida re-isolated from the pig, 10 of 10, seven of 10, and nine of 10 that were exposed to the culture in the feed, drinking water and IN respectively died of acute fowl cholera in two days with the exception of one bird that died in three days.

DISCUSSION

Since most of the animals tested were susceptible to or potential carriers of the fowl cholera organism, these experiments re-emphasized the necessity of a good sanitary program in the control of fowl cholera. The practice of feeding chickens dead of fowl cholera to other animals, or allowing domestic animals, free-flying birds or rodents to come in contact with dead or diseased birds leads to a source of infection for neighboring or replacement flocks.

In contrast to the response of the calf, sheep and pig to the highly virulent avian strain of P. multocida, Gochenour killed each of these species of animals within 24 hours with a subcutaneous injection of 0.1 ml., 0.1 ml. and 1.0 ml. respectively with a strain isolated from a buffalo in the Yellowstone National Park in 1922. The buffalo strain is serotype two and the avian strain is serotype one. Fortunately type two is no longer encountered in the United States.

It has been reported that temperatures of normal healthy mink may be as high as 108°F after they become excited or have been run around the pen in an attempt to catch them. This may have been the reason for the high temperatures in the two 24-week-old mink as they were very excited and difficult to catch.
SUMMARY

Various animals were exposed to a culture of Pasteurella multocida isolated from a chicken dead of acute fowl cholera to determine their susceptibility to fowl cholera and their potential as carriers of the organism. Pigeons, sparrows, mice and rabbits died of acute septicemia when exposed intranasally.

Rats, ferrets, guinea pigs, a sheep, a pig, and a calf failed to elicit any noticeable response to an intranasal exposure. There was a transitory temperature rise in a seven-week-old mink.

The organisms were re-isolated from the nasal passages of the calf and the pig 34 days after exposure and were still highly virulent for chickens. For this reason, other domestic farm animals may prove to be potential carriers of this disease.

One of five rats, one of two mink, and 11 of 19 mice fed viscera of chickens dead of fowl cholera developed a nasal infection, pneumonia and fatal septicemia respectively.

A calf died of acute septicemia in less than 18 hours after an intramuscular injection of the organism.

These experiments re-emphasize the necessity of a good sanitary program in the control and eradication of fowl cholera.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE
DISEASES OF POULTRY

Harry E. Goldstein, Columbus, Ohio, *Chairman*; R. A. Bankowski, California; C. H. Cunningham, Michigan; W. F. Lamoreux, California, B. S. Pomeroy, Minnesota; Henry Van Roekel, Massachusetts; J. W. Walker, Washington, D. C.; Robert Houge, Indiana; Jack E. Hanley, Florida; Fred Smith, Georgia; D. V. Zander, Washington; Harold Chute, Maine; Lee Grumbles, Texas; McK. Willis, Maryland; O. L. Osteen, Maryland; G. B. Estes, Virginia

The Committee Report for this year has again taken on a dual purpose. Last year's report deleted the abstractions relative to the latest information relative to the important transmissible diseases of poultry. Due to the great number of requests that this be part of your Committee Report, we have included that phase of this year's Committee Report.

This year's Committee continued its regulatory studies involving Pullorum Disease, Fowl Typhoid and the important problem of Mycoplasmosis.

**MYCOPLASMA GALLISEPTICUM (PPLO) ERADICATION**

Progress has been made toward eradicating infectious sinusitis from turkeys and PPLO (S-6) type from chickens.

A major step will be accomplished when a uniform, dependable diagnostic test antigen becomes available to all states. The U.S. Department of Agriculture has accepted the responsibility for the development of such a test antigen in cooperation with technical research people engaged in antigen production in the various states. This group will develop, standardize, and field test a reliable antigen, and eventually recommend a single protocol for its production.

Several turkey producing states have formal voluntary sinusitis eradication programs in effect. Such programs are proving successful and have confirmed the belief that sinusitis in turkeys caused by the S-6 type of *M. gallisepticum* can be eradicated.

Many chicken breeders have by various procedures, and combinations of procedures developed PPLO-free chicken flocks. These flocks are not being multiplied in order to meet market demands.

Representatives of the Animal Husbandry Research and the Animal Disease Eradication Divisions of the Agricultural Research Service, U.S. Department of Agriculture are formulating a standard terminology to be used for identifying flocks, counties, and states which may participate in programs for the control and/or eradication of *M. gallisepticum* infection. Upon approval of this document by the Administrator of Agricultural Research Service, it will be submitted to members of the industry, U.S.L.S.A. and elected members of the NPIP and NTIP for their consideration. Then the final document will be submitted to the industry and state regulatory officials for their consideration.
Industry must be reminded that good management and strict sanitation practices must be followed when PPLO-free flocks are grown. Additional research to further isolate and characterize strains of PPLO not of the S-6 type should be encouraged.

**MAINE SPF POULTRY FOR USLSA ANNUAL REPORT**

A Specific Pathogen Free (SPF) bird program has been in effect for about two years. A rigid set of standards for isolation and husbandry are required to conform to the program. It is a cooperative program with selected poultryman of the state; Department of Animal Pathology; University of Maine; ADPD of USDA; ADE of USDA, and the Maine State Division of Animal Industry.

A contract is signed and the poultryman agrees to conform to the rigid sanitation and husbandry requirements.¹

The first year 21,700 breeding hens were free of PPLO (S6). Besides this, there were 25 SPF broiler flocks comprising 383,190 birds. Approximately 358,890 were free of Newcastle disease and 150,490 free of infectious bronchitis. Only 35,500 in four flocks were free of PPLO. However, all of the flocks were free of pullorum typhoid, laryngotracheitis and fowl pox.

During the past year, a total of 20 SPF breeding flocks comprising 70,960 birds have been free of PPLO. Approximately 142,654 samples have been tested in the laboratory for PPLO. A method of mass testing (tube agglutination) for PPLO has been developed.

A total of 65 SPF broiler flocks, totaling 1,502,862 birds with an average weight of 2.78 lbs. at nine weeks have been processed. Forty-six flocks have been free of infectious bronchitis, 34 of PPLO and 62 of Newcastle Disease.

Evidence is accumulating that it is possible to produce poultry on a commercial scale free of the common poultry diseases.²

**Newcastle Disease**

This infectious disease continues to be one of the more important diseases in poultry as demonstrated by the amount of research that is conducted to understand the nature of the infection, methods for its prevention, control, and eventual irradiation.

A report of a severe Newcastle Disease outbreak in Puerto Rico yielded a highly virulent virus designated as strain "Ramirez" which caused lesions of the Asiatic form of the disease. Jungherr and Markham³ were able to demonstrate that the agent was antigenically similar to the North American types and could be successfully controlled with the common Newcastle Disease has also been reported as a major poultry infection in Guatemala.⁴

A number of reports on the immunogenicity of various strains of NDV and methods of their application have been investigated but unfortunately a standard criteria for evaluation of resistance to exposure are not being
The presence of a passive immunity in chicks hatched from vaccinated or previously infected dams continues to be a stumbling block in producing an active and efficient immune response to vaccination of chicks. Richey and Schmittle reported upon their studies of chicks having various levels of passive immunity, as represented by their HI antibody titers, at one day of age that were vaccinated with the B1 strain of NDV by the intranasal route. Chickens having higher levels of HI antibody at the time of vaccination showed a progressively greater drop in the HI and SN titers following vaccination and a greater cumulative mortality upon challenge at weekly intervals during the first eight weeks of life. Wills and Luginbuhl demonstrated that the yolk material of eggs laid by immune hens containing high levels of antibody when injected in two ml. quantities subcutaneously protected 80 percent of the birds against a challenge dose of virulent NDV for four weeks. The authors suggested that this procedure may be of value for protecting broilers against the disease that are sold at seven weeks of age. The importance of considering residual immunity in chicks in a revaccination program was demonstrated by Bankowski and Corstvet. The authors showed that a delay in revaccination from 32 to 39 days of age, a sufficient period to reduce the residual resistance, had a marked influence on the quality and duration of immunity to challenge when the birds reached an age of 59 and 93 weeks of age.

Interest in the killed or inactivated NDV vaccines was renewed as shown by the more recent and basic investigations to determine methods of inactivating the virus but regaining its immunogenetic characteristics. Parez believed that a formalin inactivated vaccine prepared as a more fluid product was superior to more viscid commercially prepared vaccines for inducing an immunity. Appleton, et al. concluded that formalin used under specified concentrations and conditions proved to be a better inactivating agent than beta propriolactone (BPL). The authors discussed the difference in a manner in which vaccines were prepared in the past which may explain, in part, the wide variations heretofore reported with inactivated vaccines. The authors also discussed the importance of the interval between revaccination and its influence on the quality of immunity the products were capable of stimulating. The importance of strain of virus employed in a BPL inactivated vaccine was shown in a comparative trial by Simmins and Baldwin. The mild strain "P" of NDV, when prepared as specified, proved to be as good as the more virulent "Herts" Strain F NDV in many respects resembles strain B1. A review of its characteristics and use as a vaccine in several countries in Europe, Africa, and Asia suggests that it may be more antigenic than the Blacksburg strain. Lancaster also conducted an excellent and intensive review of Newcastle disease as an infection of poultry throughout the world and the means by which it is perpetuated. Raggi and Lee employing the B1 strain of virus by the intranasal route to fully susceptible chickens at an age capable of responding to antigenic stimulus (three months old) resisted an intramuscular challenge with 200,000 ELD of GB (Texas) strain of NDV sixteen months later.
Dardiri and Yates\textsuperscript{12} compared three methods of vaccination using the recommended procedure with the B\textsubscript{1} strain as a spray and a commercially available killed product to determine the duration of immunity induced in chickens. The authors were able to demonstrate resistance to intramuscular challenge at 18 months of age that resulted in a mortality of eight to 12.5 percent. When the chickens were 12 months of age, however, a lowering of resistance of the vaccinated birds noticeably declined following vaccination with three doses of the spray type of vaccine. Similar results were obtained in a group of birds that received three doses of a killed virus vaccine that were challenged by the same methods. Unfortunately the authors did not consider a drop in egg production as an equally important sign in establishing resistance to the disease. Dixon and Torbert\textsuperscript{13} compared vaccines prepared from the B strain given in the water at the recommended intervals with chickens vaccinated with tissue culture modified strain (TCND) which was given intramuscularly. Challenge of the birds at twenty weeks and one year of age showed that the unvaccinated controls and those vaccinated with the B\textsubscript{1} strain were susceptible to each challenge but those vaccinated with the tissue culture attenuated strain resisted challenge as based on absence of mortality, respiratory and nervous symptoms.

A series of studies on the nature of immunity following Newcastle disease vaccination was conducted on a flock of chickens immunized with TCND to produce birds in various stages or levels of immunity. This was accomplished by varying the concentration of the virus in the vaccine, inducing the influence of passive immunity on revaccination, and altering the time of challenge following vaccination.\textsuperscript{7,14,15,16} The results showed that there was no correlation between signs of the respiratory tract, CNS disturbances, egg production, virus isolation and serological response in the previously vaccinated birds upon challenge. It was also found that NDV could be isolated from previously immunized, asymptomatic chickens following challenge. It was concluded that no single criterion could be used to express or evaluate the level of resistance to the disease in previously immunized chickens. It was definitely established that resistance to the respiratory tract to infection, protection against nervous signs and death and protection against a drop in egg production may react independently of each other following exposure to challenge. Furthermore there was no positive or consistent correlation between HI and SN antibody titers to the criteria mentioned above.

Because of its versatility, availability, relative nonpathogenicity for laboratory personnel, and host range, NDV proved to be a useful tool for teaching and research in numerous laboratories. Studies of the virus in tissue culture showed that the variation among strains was reflected in its behavior and ease or difficulty of propagation in various tissue culture systems.\textsuperscript{17,18,19} The pathogenicity of the lentogenic B\textsubscript{1} strain of NDV for man was again verified by a report of the infection following vaccination of chickens with the dust type vaccine. In addition to "flu-like" symptoms and a conjunctivitis, the virus was isolated from eye washings and urine specimens collected 36 and 48 hours respectively after exposure.\textsuperscript{20}
The versatility of this virus also lends itself to basic studies on the interrelationship between genetics of the host and the virus. Reta et al.\textsuperscript{21} showed that both sires and dams made statistically significant contributions to the variations in the titers of the virus grown in their eggs. Karrar\textsuperscript{22} was also able to demonstrate a natural resistance of some embryos to NDV infection. Although studies on the influence of nutrition upon the severity of a virus disease are limited, Squibb\textsuperscript{23} showed that addition of 30 times the requirement of thiamine and seven times the requirement of balanced B complex in the vitamin mixture in the feed, increased the mortality due to NDV from three to 13 percent over birds with a normal vitamin requirement. Riboflavine supplementation however reduced mortality from the disease by 10-17 percent.

\textit{Infectious Laryngotracheitis (ILT)}

The appearance of "breaks" during the past few years in flocks recently vaccinated with ILT vaccines have stimulated a number of investigators to critically re-evaluate the circumstances involved. Shibley et al.\textsuperscript{24} found marked differences in the immunizing potential of nine strains of ILT virus in cockerels previously immunized to the isolates and then cross challenged with the homologous and heterologous strains. Only two virus strains stimulated an immunity in chickens which were immune to all of the eight heterologous isolates. The authors found no real correlation between serum virus neutralizing indices and resistance to challenge. A further report\textsuperscript{25} using strain No. 146 as a vaccine applied into the conjunctival sac resulted in an immunity which resisted 1,000 embryo ELDS\textsubscript{50} of the homologous virus for 372 days. Another advantage of the strain of virus was that contact chickens placed with the vaccinates 28 days after exposure remained susceptible to challenge. The immunity elicited by this strain (No. 146) resisted an intrasinus challenge to seven of eight heterologous strains of ILT virus. Gentry\textsuperscript{26} evaluated the techniques for propagation and detection of ILT virus. Of the four methods of inoculation, viz: (a) on the chorioallantoic membrane (CAN), (b) into the chorioallantoic sac CAS), (c) through the air cell using six instead of one puncture (CM), and (d) into the CAS and by the CM routes, no significant difference in titers of the virus were obtained. The results did show, however, that end points calculated on plague formation were more consistent than those calculated on the basis of embryo mortality. The author concluded that inoculation by the SM-CAS method and using the allanotic fluid - chorioallantoic membrane mixture yielded the maximum titre and was a more effective method for detecting the virus.

In Australia, where ILT remains one of the more common respiratory diseases of fowl, significantly high degrees of immunity are obtained for at least nine weeks by exposing 10 to 12 feather follicles of one day old chicks to the commercially available products. This method is primarily for broiler chickens.\textsuperscript{27} Sinkovic\textsuperscript{28} using vaccines prepared in chicken embryonating eggs or from fresh tracheal exudates found that they were equally effective in stimulating an immunity. The vaccines are given to chickens eight to 10 weeks of age with an abrasive applicator applied to the cloacal
wall in a rotary fashion similar to the method used in the United States. There was no significant difference in the immunities produced by the vaccines but a significant difference was noted between the birds challenged intratracheally and those challenged by contact. The author concluded that it is probable that vaccination against ILT confers immunity for at least eight weeks and that the severity of "takes" cannot be used as a criterion for the degree of immunity to the disease. In another investigation Howes, et al. evaluated the immunity to ILT vaccination and attempted to determine a suitable potency standard which would remove one of the sources of failure of vaccination under field conditions. Using varying concentrations of virus the immunity against subsequent challenge via the tracheal route showed that low concentrations of virus rarely provided an immunity whereas gradual losses of immunity resulted with slightly higher concentrations of virus in a gradual transition in response to challenge from predominately lethal to predominately a mild disease. The immunity therefore was not an all or none type. From their data 90 percent of the birds acquired a complete immunity with virus concentrations of the order of $10^6$ pock forming units per ml. if the birds were vaccinated by the cloacal route and that the product and virus strain used induced detectable antibodies in the majority of the birds. The authors also suggested that it is unlikely that this standard may be valid under all circumstances. In defining minimum standards, the strain of virus, routes of vaccination, age of chickens in which the vaccine is to be employed, and antibody response to the product should be considered. Evaluation by these criteria were believed to be more accurate than the present requirements suggested in "Methods for the Examination of Poultry Biologics" that is used in this country.

Causes for "breaks" following vaccination may not be always caused by loss of potency of the vaccines. Hofstad showed that ILT was the most stable virus in a lyophilized state followed in order by fowl pox, NDV, and IBV. ILT vaccine stored at 37°C after three years showed an average log titer of 3.8 compared to 5.7 for the control virus suspension stored at 3°C.

Avian Encephalomyelitis

On March 7, 1962 avian encephalomyelitis (AE) vaccine live virus of chick embryo origin was licensed for intrastate distribution subject to written permission from proper authorities in each state of destination. The use of live virus AE vaccine has been accepted by some breeders of poultry as a control measure in its ability to prevent AE in progeny of vaccinated birds for field use. On the other hand some investigators and state authorities are reluctant to accept an unattenuated strain of virus that may become sufficiently widespread and pose a considerably greater problem in the future. Although AE is reportedly widespread in some areas Chute reported a decline in the clinical incidence of the infection in Maine during the past 10 years. In a survey conducted, only 57 percent of the flocks were shown to be immune by examination of fertile eggs for the presence of antibodies. Therefore a substantial number of flocks within the state were considered susceptible.
Although Coturniz quail have not been previously reported as being susceptible to this virus, Hill and Raymond\textsuperscript{33} were able to demonstrate that a virus isolated from chickens inoculated into this species exhibit the same symptoms and course of the disease as chickens.

**CELO and GAL Viruses**

Since AE and CELO viruses have been associated with the enteric cytopathic (CP) isolated Taylor and Celnek\textsuperscript{34} were able to isolate 45 CP agents by rectal swabs from chickens of various ages submitted for diagnosis to the University of Massachusetts. The viruses could be, by SN tests, divided into fifteen antigenic groups. One of the groups was shown to be identical to CELO virus but none were serologically related to AE. Yates \textit{et al.}\textsuperscript{35} continuing their studies with the CELO virus showed that fertile embryonating eggs inoculated prior to incubation died from the infection provided the inoculant was in high concentration. When lower concentrations of virus were used and the embryos were employed for preparation of tissue cultures, the virus had a cytopathic effect in uninoculated cultures. A detail study\textsuperscript{36} of the physical characteristic of the CELO virus demonstrated that it was 70 µ in size and more sensitive to UV light than MDV. Electromicroscopic studies suggested that the virus shares many properties with the adeno-group in size, shape, and multiplication sites. It was suggested that like the GAL virus it should be placed in the group of avian adeno-like viruses.

The pathogenesis of the GAL adeno-like virus studies revealed that the virus was excreted in the feces, saliva, and could be detected in the viscera for as long as 66 days after inoculation particularly when the antibodies were low at the time of infection.\textsuperscript{37} The virus was highly contagious and its behavior in the host resembled the viruses of visceral lymphomatosis and Newcastle disease.

**Infectious Bronchitis Virus**

According to Adler, \textit{et al.} (1962), chickens infected with \textit{M. gallisepticum} at different intervals preceding exposure to infectious bronchitis virus developed coryza, tracheitis, and airsacculitis. The chickens did not develop clinical signs. The lesions resulting from the mixed infection were indistinguishable grossly from those produced by intraperitoneal inoculation of \textit{M. gallisepticum}.

An indirect hemagglutination test for detecting infectious bronchitis virus antibodies in chicken serum using horse erythrocytes modified by tannic acid has been described by Brown, \textit{et al.} (1962). Correlation of neutralization and indirect hemagglutination indexes was indicative of the specificity of the test. The test was more sensitive at 4 C. than at 22–26 C. and the readings were stable for three to four days.

Chomiak, \textit{et al.} (1963) demonstrated that chickens vaccinated with the Beaudette culture of infectious bronchitis virus did not have respiratory signs of infection and were not protected from challenge by the virulent Massachusetts culture of the virus but neutralizing antibodies were produced. The anti-Beaudette virus serum neutralized the Beaudette virus
but not the Massachusetts virus. Anti-Massachusetts virus serum neutralized both viruses.

Testing a single dilution (1:8) of serum against a standard reference infectious bronchitis virus containing 100 ELD\textsubscript{50} per 0.1 ml. and using only four embryos per serum is satisfactory for spizootiological surveys for infectious bronchitis according to Fontains, et al. (1963). The authors state that the simplified technique permits a rapid examination of many sera more quickly than the classical technique for neutralization tests with decreased expense.

According to Hofstad and Yoder (1963), determining the rate of loss of infectivity of virus preparations after storage at 37 C. appears to give rapid indication of the stability of the virus. Whether or not predictions can be made of the loss of infectivity at 3 C. from the results obtained at 37 C. is debatable, particularly if attempted with all viruses. If the rate of loss is exponential at two temperatures, predictions can be made, but with infectious bronchitis virus, differences occur. It would be inaccurate to predict the loss of infectivity of infectious bronchitis virus at 3 C. from the results obtained at 37 C.

Mallmann and Cunningham (1963) reported that allantoic fluid from chicken embryos infected with infectious bronchitis virus contained a factor which enhanced the attachment and rapid formation of monolayers of whole chicken embryo cell cultures on glass surfaces. The factor was not present in normal allantoic fluid, cell cultures infected with the virus, or tissues of infected chickens. The cytopathic effect of the factor could be assayed quantitatively. The factor was not serologically or cytopathically identical to the virus.

The Beaudette culture of infectious bronchitis virus produced a cytopathic effect in chicken embryo kidney and adult chicken kidney cell cultures. The Wachtel culture produced mild cytopathic effect only in the kidney cell culture, whereas two different Massachusetts cultures failed to produce cytopathic effects in kidney and embryo cell cultures. A syncytial type of cytopathic effect was produced by the Beaudette culture of the virus. Inclusion bodies were not found in the cytoplasm or nucleus of the infected cells. The virus was sensitive to ether. None of the strains produced cytopathic effects in monkey kidney cell cultures.

Oshel (1963) reviewed the standard requirements for testing infectious bronchitis vaccines and the poultry biologics services at the National Animal Disease laboratory.

According to Page and Cunningham (1962), thermal inactivation of the Beaudette culture of infectious bronchitis virus was exponential with reaction rate constants related to specific temperatures. Neutralization of the Beaudette virus by anti-Massachusetts infectious bronchitis virus serum by the virus dilution method was exponential within 15 minutes to a surviving fraction of virus of less than 10\textsuperscript{-5}, or neutralization of more than 99.99 percent of the virus. Neutralization was related directly to temperature. An inverse proportionality existed between the amount of antibody and neutralization of the virus.
According to Prince, et al. (1962), feed efficiency was not significantly affected by bronchitis virus infection of chickens at 49 F. or 75 F. and ventilation rates of 3/4 and two cubic feet per minute. Feed consumption and weight gain were significantly reduced as a result of infection. Differences in mortality due to infection or ventilation rates were not significant.

An excess of infectious bronchitis virus consistently interfered with the growth of Newcastle disease virus in embryonating chicken eggs according to Raggi, et al. (1962). The time interval between administration of each virus was less important than the relative amounts of each virus. The allantoic fluid from embryos that died but that did not cause hemagglutination after inoculation of infectious bronchitis-Newcastle disease virus mixtures contained infectious bronchitis virus. Heat inactivated infectious bronchitis virus did not interfere with Newcastle disease virus. Interferon did not appear to play a detectable role in the interference of infectious bronchitis virus over Newcastle disease virus.

Vasington, et al. (1963), reported that the passive hemagglutination test in its present form is considered to be of possible use in screening anti-infectious bronchitis sera but is not considered satisfactory for determination of comparative serum titers.

According to Wintherfield and Hitchner (1962), two virus isolates (designated Gray and Holte) induced nephritis and nephrosis in chickens and mild respiratory signs similar to those produced by infectious bronchitis virus. Although these isolates are serologically different from each other, both produce neutralizing antibodies against the Beaudette culture of infectious bronchitis virus. Significant neutralization did not occur when Massachusetts, Connecticut, Iowa 97, and Iowa 609 cultures were used. There was no serological relationship with quail bronchitis virus.

The agar gel precipitin test, according to Witter (1962), is of most value for the diagnosis of infectious bronchitis virus based on increases in precipitin titer of sera collected between the third and 14th days after the onset of respiratory signs. Positive tests on single samples of serum can be used for a presumptive diagnosis.

Avian Leukosis

Sevoian, Chamberlain, and Larose (1969) have established that transmission of the lymphomatosis agent occurs via the air route. Ventilation systems from modified Horsfall isolation units containing lymphomatosis infected chicks were interconnected with ones containing susceptible chicks. The rate of demonstrable transmission was 24 of 30 chicks after 24 and 40 days of exposure. The clinical and pathological manifestations of the disease were indistinguishable from those of parenterally-induced or natural infections.

Cholera

A study made by Heddleston (1940) indicates that the existence of distinct immunogenic strains of Pasteurella multocida can account for certain fowl cholera vaccination failures. When a bivalent emulsified killed
vaccine was used, it protected against the two exposure strains for 37 weeks.

Avian Nephrosis

A new avian disease syndrome has been reported by Cosgrove. The etiology of this condition as seen in field cases has not been definitely determined. Winterfield and Hitchner have worked with two virus isolates known as Gray & Holte and found them to produce nephritis-nephrosis syndromes. Commonly referred to as Gumboro Disease, this condition may occur in two to 15 week old chickens with 10-20 percent of the flock showing morbidity. Watery diarrhea, increased urate excretion with soil ing of the vent feathers, anorexia, trembling, depression, prostration and death may be observed in birds with this condition. A mortality of about five percent seen in two to five week old birds. This disease usually lasts five to seven days.

Internal changes of dehydration, hemorrhages in the leg muscles, increased mucus in the intestine, occasional small liver infarctions, enlargement of the bursa of Fabricius with an occasional white core, and nephrosis are seen. The enlarged, urate distended, kidneys and the enlarged bursa of Fabricius are particularly characteristic of this disease. A lowered blood calcium value of three to five percent as compared to a normal of eight to 10 percent has been observed.

Hemorrhagic Anemia Syndrome

Work on the etiology and development of this condition by Forgacs, Koch, Carll, and White-Stevens indicate that certain fungi can produce a hemorrhagic anemia syndrome similar to that seen in field outbreaks. Additional work by Forgacs, Koch and White-Stevens indicate the value of certain antifungal substances in the feed where it will continue to be present even when the feed has spilled into the litter. In subsequent work, the value of eight hydroxyquinoline as an antifungal agent was observed.

Equine Encephalomyelitis

In August and September of 1963, Eastern equine encephalomyelitis was isolated from chunker partridge on five different farms in entirely separate areas in Florida. The agent was isolated in embryonated avian eggs. Neutralization tests were used to identify the presence of the Eastern strain and HI tests were used to survey the associated bird populations.

Several of these farms also had pheasant and quail on the premises, but, at this time, only the young chukars were visibly affected. Serology indicated a past experience with EEE in the quail on one of these farms.

Laryngotracheitis

Shibley, Luginbuhl and Helmboldt have worked with a strain 146 ILT virus (obtained from Dr. M. S. Cover, University of Delaware) and have been able to produce a vaccine which can be administered in the conjunctiva. Some degree of swollen eyelids were noted five to seven days
post-vaccination. All birds appeared normal by the 11th day post-vaccination. Adverse effects were not detected during safety and potency tests. Cockerels vaccinated at 13-weeks of age remained normal when exposed 372 days post-vaccination with intrasinusoidal challenges of 1,000$\text{s}_0$ of a heterologous ILT virus.

Gelenxxzel and Marty$^{46}$ have worked with a laryngotracheitis virus, ASL L-6 strain. The strain was adapted to a primary monolayer avian origin tissue culture system and systematic passage of the strain in cell culture resulted in two basic changes in viral characteristics. Atypical lesions resulted on the chorioallantoic membranes of chicken embryos, and the virulence for susceptible chickens was significantly decreased. Initial studies on strain stability demonstrated that the modified virus retains its distinguishing characteristics through two back passages in embryos and chickens.

Desiccated vaccine prepared from the modified virus provided good protection against virulent challenge of chickens vaccinated either ocularly, intranasally, or through the vent. Similarly, good immunity against a variety of challenge strains was obtained when the vaccine was applied ocularly. On the other hand, spray and water administration of the modified virus resulted in poor immunity as assessed by challenge. Further studies on application of the vaccine showed that birds two weeks or older develop good immunity, whereas those younger than two weeks do not develop dependable flock protection. Laboratory experiments indicated that a strong durable immunity lasting for at least 22 weeks develops in birds vaccinated at two to six weeks of age. Also, it was demonstrated that the vaccine virus spreads to non-vaccinated contact controls, but no deleterious effects of virus spread were noted in the susceptible chickens.

The following resolution was drawn up and read at the New England Veterinary Association Meeting held at Pike, New Hampshire, September 22-25, 1963.

The Committee has continued its efforts and study toward an eradication program for Pulmonary disease and Fowl Typhoid. These efforts have been directed to be sympathetic with the desires of the poultry industry, the National Plans Program, and the regulatory agencies.

In view of the low incidence of pullorum disease and fowl typhoid in the New England states for the past ten years and since an appreciable number of these outbreaks originated from outside of New England, the State Animal Disease Regulatory Officials and poultry pathologists in the respective area are of the opinion that steps should be taken to eradicate these two diseases.

However, it has been reported that the National Poultry Improvement Plan is contemplating proposing a partial testing program at the next general conference of the Plan participants in 1964. It is the professional opinion that partial testing program as outlined is incompatible with the concept of eradication since it would jeopardize the progress made in flocks and areas where these diseases have been eliminated.

Furthermore, this matter should be discussed with the Administration of the Agricultural Research Service, USDA for clarification of direction to eradicate the above diseases.
REPORT OF COMMITTEE

This report should be submitted to the Chairman of the Committee on Transmissible Diseases of Poultry, of the United States Livestock Sanitary Association for appropriate action.

Your Committee recommends the following for consideration of the Executive Committee:

(1) The Committee strongly recommends that ARS, ADE give serious consideration to the systematic national eradication of pullorum disease and fowl typhoid, by areas utilizing the Proposed Uniform Methods and Rules as recommended by the United States Livestock Sanitary Association.

(2) The Committee recommends that the efforts of ARS, ADE continue towards developing a standardized antigen for mycoplasma and gallisepticum to continue the efforts toward more uniform terminology and standard procedures.

(3) The Committee recommends that the executive committee appoint a subcommittee comprised of members of the Tuberculosis Committee and Transmissible diseases of Poultry Committee to study the problem of Avian Tuberculosis in relation to the National Tuberculosis Eradication Program.

(4) Avian leukemia has become one of the most serious economic problems of the poultry industry; this committee recommends that ARS provide sufficient funds to expand and initiate research projects at East Lansing to cope with and to support other research groups in their activities and efforts to elucidate avian leukemia complex.

(5) The Committee recommends that each livestock sanitary official of our state give serious consideration to the recommendations of the report of the Salmonellosis Sub-committee of the Committee of Infectious Disease of cattle, relative to the sanitation guidelines for processors of animal poultry, and fish by products, to aid in the control of Salmonellosis.

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THE VETERINARIAN'S RESPONSIBILITY
IN THE USE OF PESTICIDES

R. J. Anderson, D.V.M.*
Washington, D.C.

I am glad to have this opportunity to talk with friends who share in the united effort to safeguard animal health in this country.

Today, I want to discuss a matter that concerns not only the health of the Nation's livestock but also the well-being of our crops and forests, our fish and wildlife—and, indeed, of you and me and 190 million neighbors of ours.

You are well acquainted with the necessity for safe, effective, economical methods of controlling pests—which include thousands of kinds of insects, diseases, weeds, nematodes, and parasites. You know, too, that we have been meeting most of these threats with a wide variety of chemical pesticides.

Such materials have had a lot to do with helping farmers supply us with more high-quality, reasonably priced food and fiber than any people ever enjoyed before. Pesticides have also contributed to human health and comfort by providing ways to control insects that attack man or transmit diseases to him.

Now, the American public is being told by some people that these pesticides are poisoning the environment, destroying beneficial forms of life, and threatening the health of human beings.

It's true, of course, that many pesticides are powerful substances, and some can be extremely dangerous. This also applied to many present-day drugs, chemicals, and machines.

In judging the risks associated with the use of pesticides, let us always keep clearly in mind this fact: some of the nation's finest scientific competence and judgment go into developing these materials and regulating their sale. Both effectiveness and safety receive the most serious consideration. I am confident that these materials are safe to use—if they are used according to the warnings and cautions on the label.

Here, I believe, is a challenge for each of us: we must do everything we can to see that pesticides are used safely. Misuse can result in a variety of hazards, particularly to human health. Misuse can lead to seizure of farm products contaminated with illegal residues. And every incident of accidental or careless misuse of pesticides is bound to add to the pressure for suppressive legislation.

You know where some of the potential problems are:
In homes and gardens, careful attention must be given to choosing the right pesticide, applying it exactly as the label prescribes, and storing it properly after use.

*Deputy Administrator, Agricultural Research Service, U.S. Department of Agriculture.
On farms and ranches, many of the problems are similar but on a larger scale. To avoid residues in food or to keep them well within tolerance limits, special attention is required to such matters as withholding cattle from treated pasture prior to slaughter, controlling flies in dairy barns without leaving residues in milk, and timing applications on crops at the prescribed interval before harvest. Hazards to wildlife and beneficial insects must be avoided or minimized by using insecticides sparingly and only where they are needed, and by avoiding contamination of streams and other bodies of water.

These are matters that you understand—because of your training, and because of your experience in cooperative control programs. You are in position to use this understanding to good advantage, not only in your own operations but also in your day-to-day contacts with the public. You talk with a lot of farmers. You meet the private practitioners on whom many farmers depend for guidance. In addition, you are called on to talk to local groups from time to time. You are expected to know the answers, and you are listened to with respect. Few people in this country have the opportunity you have to put across the message on safe use of pesticides.

As I see it, we Federal and State veterinarians have two major responsibilities here: one is to keep informed and pass the word along; the other is to conduct our own operations safely.

Let's look first at the matter of keeping informed. Our education on pesticides should be based, of course, on solid training in colleges of veterinary medicine. I hope that every school is now covering the subject of pesticides thoroughly, and emphasizing to students the responsibility they will bear as veterinarians for seeing that these materials are used with care.

We need to follow up our education by reviewing the professional journals regularly and by keeping abreast of information available from the United States Department of Agriculture and other Government agencies concerned with the use of pesticides. The Department has tried to help by furnishing you such materials as the report of the President's Science Advisory Committee and the statements by Secretary Freeman and Doctor Clarkson before the Subcommittee on Reorganization and International Organizations of the Senate Committee on Government Operations.

Since the United States Department of Agriculture has some of the nation's most important assignments in the area of pest control, I want to tell you something about the determined effort we are making to tighten up our operations on these jobs.

One Department assignment is to conduct research to develop better methods of providing protection against pests.

Chemical pesticides have long been the main weapon, and the United States Department of Agriculture has had a big hand in developing them, in cooperation with industry and the State experiment stations. The Department's work in identifying useful chemicals and devising ways to apply them has helped to bring many new pesticides into use, especially during the last two decades.
In this work, we have always given attention to minimizing the possible harmful effects from pesticides.

For example, cooperative work by our entomologists and veterinary toxicologists at Kerrville, Texas, over the last 12 years has taught us a great deal about the toxicity of insecticides to animals. A chemical may control a parasite of livestock or an insect on a forage crop—but how will animals that come in contact with the material be affected? We explore both the acute and long-term effects as well as the fate of the original material and its breakdown products. This work is also giving us new knowledge on symptomatology, which is vital in helping field veterinarians differentiate between a case of pesticide poisoning and the onset of a disease.

Department scientists were among the first to recognize the problem of insecticide residues in meat and milk in the late 40's, and we are still continuing the search for safer materials.

But the United States Department of Agriculture research on pest control has been far broader than the development of chemical weapons.

We have made many important contributions in devising effective cultural practices, as well as in breeding crop varieties that resist damage by various pests. The United States Department of Agriculture pioneered in biological control—fighting pests with natural enemies such as insects, bacteria, fungi, viruses, and protozoa. We believe such methods hold further promise.

Our scientists are also pushing forward in the development of imaginative new approaches.

One of the most spectacular is to sterilize insects with gamma radiation and release them in great numbers to promote their own destruction. Many of you have first-hand knowledge of how effective this method is against the screwworm. Preliminary tests of the sterility principle against several other important insects have given encouraging results. We have now discovered that some insects can be sterilized with chemicals, which opens up the possibility of controlling such pests as flies, mosquitoes, and stable flies by inducing sterility in the natural population.

Other interesting approaches to insect control are based on the attraction of insects to host plants and animals, to the opposite sex, and to lights, sounds, and other types of radiation. We hope to make increasing use of attractants to draw insects to places where they can be treated with insecticides or sterilants, or mechanically destroyed. There seem to be especially promising possibilities in isolating and synthesizing the powerful sex attractants that have been discovered in some insects. At the new fly laboratory at Beltsville, a team of scientists has begun an exhaustive study of light traps and other physical methods of controlling flies that are most objectionable on dairy farms.

One of the outstanding achievements of recent years is the development of a systemic insecticide to control cattle grubs. The job is now as simple as putting this material in the feed. Department scientists have also had encouraging results in attempts to develop systemics that are effective against the sheep nose bot. Incidentally, when certain systemics are fed for grub control, enough insecticide seems to pass through into the
manure to prevent the breeding of flies. This apparent side-benefit may be confirmed by work now underway.

Another promising line of work that Department scientists are now beginning to emphasize is control of parasites in livestock through the use of vaccines, special management, and sanitation. This approach would eliminate the possibility of residues from chemical treatments and avoid the establishment of resistant strains of these pests.

In all the research areas I have mentioned, we are seriously handicapped by lack of fundamental knowledge about the organisms and material we are dealing with. So our scientists are moving as rapidly as possible to expand their basic investigations in such areas as the life processes, biology, and behavior of insects. Work like this will provide the principles for more effective pest control.

As you can see, the Department is doing a great deal of work on developing methods that do not require the use of conventional pesticides. A major shift of emphasis on entomology research was begun in 1955—eight years ago—in response to the growing resistance of insects to chemicals and the widespread appearance of low-level residues. Today, two-thirds of our total research effort on insects is devoted to such matters as biological control, highly specific chemicals, and studies of a basic nature.

Another important and even broader job assigned to the United States Department of Agriculture is to register all the pesticides sold interstate in this country.

The Department passes on the effectiveness and safety of a tremendous range of products—Insecticides for ticks...herbicides for cotton...fungicides for wood...sterilizers for surgical instruments...to mention only a few. In all, we have registered some 50,000 formulations based on more than 500 individual chemical compounds.

To protect the public from potential hazards, the United States Department of Agriculture requires the manufacturer to submit exhaustive data on any new pesticide offered for registration. There must be detailed and convincing test results showing that the product will give effective pest control under a variety of conditions. Extensive toxicological studies are required to convince us that the precautions on the proposed label will be adequate to safeguard the public. And when the product is used on foods, detailed chemical analyses of any residues must be submitted.

It's likely to take three to five years of exacting scientific work to bring together the proof of safety and effectiveness required by USDA. Applications for registration may include hundreds of pages of charts, formulas, and text setting forth the results of the testing the new chemical has undergone.

These data are evaluated by the Pesticides Regulation Division of the Agricultural Research Service. Here we have a highly competent staff of scientists, including pharmacologists, entomologists, bacteriologists, chemists, biologists, and plant pathologists. In addition, we consult on questions of safety with expert authorities in other scientific institutions and Government agencies, such as the Food and Drug Administration, the Public Health Service, and the Fish and Wildlife Service.
The Department provides a further important protection to the public by stringent control over the labels used on pesticides. We must know exactly what each label will say and be satisfied that it gives adequate directions and warnings for safe use of the product.

I particularly want to emphasize that the Department keeps this registration process under continuing review in the interest of safety.

As a matter of fact, the United States Department of Agriculture took the initiative in proposing the legislation that established these safeguards back in 1947. We cooperated in extending the coverage to additional materials in 1959. And we have now recommended that the law be tightened up by eliminating a provision that permits an applicant to go ahead and sell his product by registering it "under protest" when registration has been denied by the United States Department of Agriculture.

Recently, the Department asked a panel of outstanding specialists to look over our registration operation and help us determine what changes, if any, need to be made to fully protect the public. This group included representatives of Public Health, Food and Drug, Fish and Wildlife, the Federal Trade Commission, State Departments of Agriculture, and the consuming public.

The views of this panel are reflected in the Department's recent proposals to improve pesticide labels. Warning statements and directions for safe use of the material must now be prominently placed on the front panel of the label. This information must be printed in legible type, and written so it's easy to understand.

The Department's concern over labels bears on a very important point:

Ultimately, the safety of pesticides depends on all of us who use them. The United States Department of Agriculture has stressed this point for years. Farmers and ranchers, shippers and handlers, housewives and gardeners have been warned to use pesticides with care. This reminder has gone into more than 30 million copies of popular publications distributed by the United States Department of Agriculture over the last 10 years to help people control insects, diseases, and weeds.

We are now putting new emphasis on safe use of pesticides through publications, posters, press releases, radio, and television. I hope you have already seen and heard the spot announcements sent to every television and radio station in the country. A new motion picture, "Safe Use of Pesticides," has been produced in cooperation with the Department of Health, Education and Welfare and is now available in every State. You should have many opportunities to use this film, as well as the leaflets and posters.

The message in all of these materials is summed up in a simple slogan: "Use Pesticides Safely—Read the Label."

The Department carries out still another important assignment, one that I certainly don't need to explain to this group. I am referring to the part we play—along with cooperating States—in directly combating several of the most dangerous pests that threaten this country.
Events of the last year or so have revealed a serious lack of public understanding of the basic facts in this area—facts that are brought home to you daily as you deal with sick and infested herds and flocks.

You know why it's important to maintain a strong line of defense against foreign pests at our air, sea, and border ports of entry. . . why it pays to eradicate a pest when we can, rather than try to live with it. . . why we must try to hold down losses by confining and controlling some pests until research can give us the weapons for eradication. You know eradication works because you see it working every day. You know, too, that these measures greatly reduce the use of pesticides in the long run.

This positive approach in dealing with agricultural and forest pests is clearly spelled out in a policy statement recently issued by the United States Department of Agriculture. I would like to insert that statement in the record of this meeting at the conclusion of my remarks. I hope you will miss no opportunity to tell this story.

Cooperative campaigns are now being waged to eradicate or control 23 crop pests and 12 pests of livestock.

Naturally, such programs sometimes call for the use of pesticides. In using these chemicals—just as in developing and regulating them—we give safety full consideration. As you are well aware, operations are planned to protect workers, people living in treated areas, crops, livestock, fish and wildlife, and anyone who might later come in contact with residues. Leaders of these programs are now re-checking field instructions to make sure that they are adequate.

All United States Department of Agriculture pest-control campaigns are under review by the new Pest Program Evaluation Group established by the Department. This Group is determining whether any program needs to be modified in any way, and will serve on a continuing basis to make sure that no possibility for improvement is overlooked.

These measures reflect a long tradition of concern for safety in the United States Department of Agriculture. It was this tradition that caused the Department to take the initiative more than two years ago in establishing the Federal Pest Control Review Board. That Board, which includes members from several agencies, must give its approval to all pest-control programs carried on by the Federal Government. The result is that these programs are conducted with proper consideration for every national interest, including agriculture, wildlife conservation, and public health.

This discussion of cooperative pest-control campaigns brings us to the other major responsibility of State and Federal veterinarians in regard to the safe use of pesticides. Along with keeping informed and informing others, we must also conduct our own operations with the utmost concern for safety.

As representatives of government agencies...with the facts about safe use of pesticides readily available to us...we are particularly vulnerable to public censure for any violation. Today, the climate of opinion is so sensitive that we cannot afford a single mistake. One careless accident can bring an avalanche of criticism that may well affect all use of pesticides.
The point I want to make is that careful planning of these cooperative campaigns must be followed up with careful execution. We must take no chances on such matters as using protective clothing and respirators when needed. . . avoiding streams and pastures in spraying operations. . . making sure animals are in good physical condition for treatment. . . properly cleaning vats and disposing of dipping solutions. . . and so on down the line.

There's another aspect of these campaigns that calls for special attention at the local level. I urge you to use every means of enlisting public support for your operations, including those where pesticides are involved. Make full use of meetings with people in the area, assistance from the Extension Service and local organizations, and the interest of press, radio, and television in your activities. See that the community knows what your program is, how it will affect the public, and what people should do. Public support can make all the difference between the failure of a program and solid success.

In closing I want to emphasize that safe use of pesticides is not a Federal problem or a State problem—it's our problem. And everyone needs to know. By keeping informed and informing others. . . by conducting our own operations safely. . . we can make a contribution of lasting value to the health and well-being of people everywhere.

UNITED STATES DEPARTMENT OF AGRICULTURE
POLICY IN DEALING WITH AGRICULTURAL AND FOREST PESTS

The Department of Agriculture has broad authority under Federal law for developing and carrying out programs to prevent, retard, eradicate, control, or suppress destructive insects, plant and animal diseases, and certain other agricultural pests. Responsibility for the exercise of this authority has been assigned to the Department's Agricultural Research Service and Forest Service.

These agencies conduct programs against pests in accordance with the Department policy outlined in this statement. Included are cooperative programs carried out jointly with interested States, local government agencies, other organizations, and individuals. All programs conducted on non-Federal lands must meet the requirements of State or local law, as well as Federal law.

The Department's efforts to combat pests are concentrated primarily on (a) those of foreign origin that have not spread to all the areas of the United States in which they could cause damage, and (b) native ones capable of sudden extensive outbreaks not controllable by local action, which may threaten the nation's agriculture or its forest resources.

Department policy in dealing with these pests is summarized as follows:

1. GENERAL. All pest prevention, control, and eradication programs are based on the most up-to-date scientific and technical knowledge
RESPONSIBILITY IN THE USE OF PESTICIDES

available. Programs involving any substantial use of pesticides are reviewed by the Federal Pest Control Review Board to insure that public health and other national interests are protected. The public is kept informed of program activities, both on a national basis and in local areas affected. Research and methods-improvement work is conducted to develop more effective and economical means of combatting pests and to assure continued safety of the programs.

2. SAFETY OF PUBLIC HEALTH AND WILDLIFE. Special measures are taken in all programs to avoid potential hazards to public health or welfare. Possible effects on fish and wildlife are given careful consideration with a view to eliminating or minimizing damage to these values.

3. DEFENSE AGAINST FOREIGN PESTS. The entry of destructive foreign pests is prevented to the maximum extent possible by thorough inspection of incoming materials at sea and air ports and border crossings.

4. ERADICATION. Efforts are made to detect and immediately eradicate incipient infestations of significant pests that do gain entry. Experience in this and other countries has clearly demonstrated that it is in the public interest to eradicate incipient infestations and infections than to permit their establishment.

5. CONTROL AND SUPPRESSION. When effective means for eradication are lacking, destructive introduced pests are confined if possible within the areas where they have become established. Efforts are made to prevent and retard their spread and, when feasible, to reduce their numbers in infested areas.

Pest control and eradication programs conducted by the Agricultural Research Service and the Forest Service illustrate the above policy in action. For example:

Port and Border Inspection. Many of the world's most destructive pests have been kept out of this country, and a number that got past our port and border guards were discovered and quickly eradicated. Foreign pests that continually threaten this country include some 180 species of insects destructive to agricultural crops and forest trees, and such feared livestock diseases as rinderpest, African swine fever, and foot-and-mouth disease. Among plant pests alone, there are an estimated 20,000 kinds that do substantial damage in foreign countries but have so far been kept out of the United States. A continuously alert inspection system maintained by the Department is a vital first line of defense against these potential invaders. The United States Department of Agriculture plant and animal quarantine inspectors halt a shipment containing some serious foreign pest every 16 minutes on the average, day and night the year 'round.

Eradication. A serious agricultural or forest pest new to this country, and detected while it is still confined to a relatively small area on private or public lands, is subject to immediate eradication if effective methods for doing the job are at hand. Eradication eliminates the need for long-term control measures, which could well require the use of increasing amounts of pesticides over an expanding area year after year, in addition to costly losses.
While efforts to eradicate a newly introduced pest are underway, domestic Federal and State quarantines are imposed to prevent spread of the pest to new areas. Also, available research knowledge and other measures are applied to reduce damage in the infested area as much as possible.

Recent successful eradication programs against the Mediterranean fruit fly in Florida and the Khapra beetle in several areas of the country are examples of this kind of effort. Unfortunately, however, effective means for eradication are not always available. This was the case with the witchweed pest of corn and other crops found a few years ago in North and South Carolina and the soybean cyst nematode discovered in the Midwest. Such pests must be dealt with at the outset by control methods.

Control. The basic measure taken against agricultural and forest pests that have become established in sizeable areas of the country but have not spread throughout their full ecological range is to establish joint State-Federal quarantines to regulate the movement of materials that might carry the pest into uninfested areas. These domestic quarantines are maintained by the Department of Agriculture and State regulatory agencies concerned. At the same time, cooperative programs are developed to reduce populations of the pest within the generally infested area. These programs may employ various cultural and biological practices, or pesticide applications.

In most cases, also, control programs involve eradicating limited infestations of the pest outside the area of general infestation. Eradication of such outlying infestations has proved both possible and beneficial, in spite of the continuing threat of reinfestation.

Such control procedures have been successful against the gypsy moth, Japanese beetle, white-fringed beetle, European pine shoot moth, and other pests.

Combined Control and Eradication. With some long-established pests, it has been possible to combine control and eradication measures for a gradual roll-back, leading to total elimination of the pest from the country or a substantial reduction in the extent of the infestation. This can be done only when research develops safe and effective eradication methods.

The current programs against the imported fire ant in the South and the screwworm pest of livestock in Florida and the Southwest are examples of this combined control-and-eradication approach. During the last 70 years many serious pests have been eradicated from the United States, including cattle tick fever, foot-and-mouth disease, Hall scale of fruit crops, Khapra beetle, Medfly, and several others.

Suppression. A number of pests widely established in the United States require a different approach. They are chiefly insects that are subject to a considerable degree of natural control, or that can be dealt with on a local basis. However, some of these pests are periodically capable of sudden outbreaks that cannot be locally controlled. Included in this group are grasshoppers, Mormon crickets, bark beetles, and forest defoliators, which cause widespread and costly damage to range grasses, cultivated crops, commercial and woodlot timber, and shade trees.
Dealing with them requires periodic suppressive measures, usually undertaken in cooperative programs which involve use of pesticides. Key to the success of these programs is an alert survey and monitoring system which gives advance warning to the location and seriousness of potential outbreaks.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH


USE OF PESTICIDES

In view of the tremendous publicity created by Rachel Carson's book "Silent Spring" the Public Health Committee agreed that we should place high emphasis on this subject. Under our sponsorship, Dr. R. J. Anderson, Deputy Administrator of the Agricultural Research Service, has presented a fine paper entitled "The Veterinarian's Responsibility in the Use of Pesticides."

In addition, the Committee has reviewed the report on "Use of Pesticides" prepared by the President's Science Advisory Committee, published on May 15, 1963. This report is "must" reading for members of the United States Livestock Sanitary Association in our opinion.

It contains brief, but concise information on gains from the use of pesticides, the hazards of pesticides, pest control without chemicals, the role of government in pesticide regulations, and recommendations. The latter are directed "to an assessment of levels of pesticides in man and his environment; to measures which will augment the safety of present practices; to needed research and development of safer and more specific methods of pest control; to suggested amendments or public laws governing the use of pesticides; and to public education."

The Public Health Committee supports these recommendations which we understand are now being implemented by the Departments of Health, Education and Welfare, Agriculture and Interior.

Of special importance to us is the amplification of research resources by stimulating training and basic investigation in the fields of toxicology and ecology. Training grants, basic research grants, and contracts to universities and other non-governmental research agencies funded by the Departments of Agriculture, Interior, and Health, Education and Welfare will stimulate this research.

Of equal importance are the recommendations to strengthen public laws on pesticides. These include the elimination of "protest registration" (with public hearings for protection against arbitrary decisions); official registration numbers required on the label of every pesticide formulation; clarification of the intent of the Federal Insecticide, Fungicide, and Rodenticide Act to protect fish and wildlife by including them as useful vertebrates and invertebrates; and to provide funds to evaluate the efficiency of the programs and promptly publish the results of these studies.

The Public Health Committee urges support of these recommendations. We further urge the membership to resist the attempts of overzealous...
uninformed or partially informed individuals who promote legislation which actually may be of little or no value in the protection of human or animal health but may result in great harm to agriculture.

SALMONELLOSIS

Human salmonellosis continues to be an important public health problem. The reported incidence over the past decade has revealed a notable increase. Part of this increase results from better reporting; however, there is a real increase which depicts a greater risk of exposure. Of the total isolations reported, the greatest number is found in the one to five age group. The mortality rates are low, being under one percent of reported cases.

Investigation of outbreaks, generally of the food-borne type, reveals poultry and poultry products as the chief sources of human infection.

Although the animal incidence of salmonellosis is difficult to determine, the number of laboratory isolations serotyped gives an indication of the species most frequently involved. The frequency of isolation by species reported to the Salmonella Surveillance Unit shows avian species comprising 40-60 percent, swine 10-20 percent, cattle five to 10 percent, with the remainder from a multitude of vertebrate species and environmental sources. The most common serotypes identified from human and animal sources are similar. The frequency by which both types appear in animals and humans illustrates the importance of animal infections contributing to the contamination of food.

The Communicable Disease Center, Salmonella Surveillance Unit, is now receiving reports of human incidence and epidemiological information on outbreaks from all of the states. Non-human isolations are likewise received from the typing centers and utilized in defining patterns of disease concentration by source and area. It seems apparent that epidemiological studies will continue to implicate the animal sources of infection resulting in recommendations to establish control programs at the animal host level as well as improvement in the processing of food products.

There is an urgent need for federal and state agencies responsible for animal disease control to assist in development of programs that will prevent the spread of salmonella infection in poultry and livestock. Efforts that have been made by the USDA to control the contamination of rendered products used in animal feeds should receive additional support for further action. There is a need to support and encourage research activities that will be required to develop sound control programs. Epidemiological investigations should be conducted to determine the existing relationships of animal infections with sources of exposure. Both the public health significance of animal infection and the economy of the agricultural industries which may be suppressed by the loss of efficiency in animal production and consumers rejection of commodities will necessitate increased efforts to alleviate the problem of salmonellosis in animals.2
An epidemic of Equine Influenza, caused by a new type A influenza virus, was evident from February through June of this year, and appeared in all parts of the United States. Investigations were conducted by the Communicable Disease Center to: (1) observe the genesis and spread of epidemic influenza; (2) gain understanding of the relationship between human and animal influenza and, (3) determine if this virus was causing illness in man.

In spite of numerous reports of concurrent illness in man and horses all thorough investigations of these have uncovered some other virus or failed to fully incriminate the A2 equine virus as the cause of disease in man.

Historical records refer to epidemics of "influenza" in horses in the 14th century. However, the first virus was recovered in 1956 and designated as A1 equine/Prague/56. There is serologic evidence indicating the presence of this virus in the United States as early as 1957.

The agent responsible for the current epidemic is also a type A influenza virus, but of a previously unknown subtype. It has been designated as A2-equine.

The disease appeared in Miami, Florida, in January of 1963. The first horses ill were apparently thoroughbreds imported from Argentina by air. Since that time many thousands of horses in racetracks and elsewhere throughout the United States have contracted the infection.

The normal clinical signs are coughing, fever of 102°F to 105°F, and anorexia. The uncomplicated illness lasts from three to 10 days. Morbidity rates reached 90 percent in some outbreaks with mortality rates at or near zero percent.

Investigation of the current outbreak and its public health implications are continuing.

NATIONAL ZOONOSES SURVEILLANCE PROGRAM

The Veterinary Section of the Communicable Disease Center, Public Health Service, is expanding the National Zoonoses Surveillance Program. It will be based on existing animal and human morbidity and mortality reporting systems. No attempt will be made to establish a new data collection system. Available data will be collected and tabulated and reports issued quarterly and annually.

The Public Health Committee wishes to call the attention of the Association to this program. To support this activity, any official or organization which compiles any data on animal morbidity or mortality or the occurrence of any zoonotic disease in man should have these data sent to:

Chief, Zoonosis Surveillance Unit
Epidemiology Branch
Communicable Disease Center
Atlanta, Georgia 30333
Excerpts from the statements by Dr. Donald Chadwick, Chief, Division of Radiological Health, U. S. Public Health Service, Department of Health, Education and Welfare, and Dr. M. R. Clarkson, Associate Administrator, Agricultural Research Service, U. S. Department of Agriculture, before the Subcommittee on Research, Development and Radiation, Joint Committee on Atomic Energy, provide information on the philosophy and requirements of countermeasures against radioactive fallout. Related research now being conducted is also summarized.

Dr. Chadwick's statement included the following:

Basic to the considerations involved in decisions as to when to take protective measures against environmental contamination is the nature of the problem of developing radiation protection criteria and standards in general. This matter has been considered in reports of the Federal Radiation Council and extensively in testimony in previous hearings before this Committee. Fundamental is the hypothesis that any amount of radiation exposure involves some risk in exposed population groups. This has been accepted by the Federal Government as a basis for radiation protection standards. Furthermore, it is assumed that the risk generally increases proportionally with the dose. The establishment of protection criteria, therefore, involves the acceptance of a certain amount of risk. The amount of risk accepted depends upon the reasons for accepting the risk or the alternates which are available for avoiding it.

In the usual situation of normal peacetime operations, control is exercised by limiting the amount of radioactive materials which can be released to the environment. The judgment as to how much should be released, and thus how much risk the population should accept, involves limiting the release to a level which meets the criterion that the extra effort required to effect further reductions clearly outweighs the reduction in risk which would be accomplished by the reduction in exposure.

A different kind of a judgment is involved when the radioactive materials are already dispersed in the environment and reduction in exposure must be accomplished by some interruption of the pathway from the environment to man. Here the total impact on our society of any available measure for reducing exposure of man must be weighed against the reduction in risk which the measure will accomplish.

A great deal of attention has been directed to the evaluation of the possible risk to exposed population groups as a result of exposure from radioactive materials in the environment. Less attention has been directed to an evaluation of available protective measures and the potential impact which would be accompanied by the application of these protective measures. Clearly, decisions as to when to employ protective measures depend upon careful evaluation of the possible risk of deleterious radiation effects which the measure might reduce or eliminate. Similarly, there must be
careful evaluation of the difficulties which would be encountered in the application of the measure. These difficulties vary widely, not only from one protective measure to another, but also in the case of certain measures, from one geographic area to another and from one season of the year to another.

A few examples might help to clarify this point. One measure, the effectiveness of which has been demonstrated in studies by the Public Health Service and the Department of Agriculture, is the shifting of dairy cattle from pasture feeding to stored feed in order to reduce the radioiodine content of milk. Certainly the impact of this measure would be less than that of the widespread condemnation of contaminated milk supplies. Furthermore, the difficulties of applying this measure vary widely both geographically and seasonally. In some areas dairy cows are fed stored feed essentially the year around. In these areas this "protective measure" is always in effect. At the other extreme are areas where little if any stored feed is used ordinarily and dairy cows graze at pasture the year around. Stored feed, therefore, may not even be available locally and might have to be shipped in. In the rest of the country, the difficulty of shifting to stored feed would depend upon such factors as the amount of feed available, the geographic area covered, as well as the length of time during which stored feed would need to be used. The latter point is of some importance because of the usually very acute nature of iodine contamination episodes. In these situations a relatively brief period on stored feed could cut off the peak of radioiodine contamination of milk before it was reached.

The latter point also emphasizes another important aspect of protective measures against radioiodine. Because of the very acute nature of iodine contamination, action to reduce exposure must be taken with great promptness in order to be effective. Any significant delay in reaching a decision as to whether protective measures should be used may amount, in effect, to a decision not to take such measures, as the period in which they would be effective may well have passed.

Another measure referred to in a report prepared by the Salt Lake City and Utah State Health Departments in October 1962 is the diversion of milk with high radioactive iodine content to manufactured processes to permit decay of this radionuclide. The impact of this measure would presumably depend upon the proportion of a milkshed contaminated with relatively high levels compared to the proportion which is surplus in terms of fresh fluid milk and would in any case go to manufactured processes. In any given situation it might simply involve a redistribution of supplies going to the two uses.

It is apparent from the characteristics of the nuclide and from surveillance data, that the problem of radioiodine from fallout is sharply circumscribed by time and locality. There can be great variation within different short time periods and from the same periods in the amount of contamination in milk from herd to herd and milkshed to milkshed and between states and regions. Furthermore, most of the reasonably effective protective measures that are currently available require coordinated
actions on a local, regional, state or interstate basis by a variety of governmental, agricultural, and industrial groups. Because of the acute nature of radioiodine incursions, protective actions must be applied swiftly if they are to be effective in reducing exposure. Thus local and state resources and capabilities in the field of radiological health, the status of protective measure planning, and the degree of cooperation that can be expected from farmers and the dairy industry are matters of considerable importance.

In the light of the characteristics of the nuclide, the potentially available protective measures and the long-term rather than acute nature of the exposure problem, the strontium 90 situation is strikingly different. There are fewer and less striking differences in concentrations of strontium 90 in milk and other foods over short time periods and between adjacent localities and regions. Processed milk, cereal grains, wheat and canned foods are distributed nationally rather than near the area of production. Thus the problem of strontium 90 contamination of foods obviously is national rather than local. The impact of potentially useful protective measures is another factor which would seem to involve national action rather than local, state, or regional interstate efforts.

Thus the question "When should protective measures be taken?" is meaningful only in relation to the radionuclide in question, the specific measures available and the over-all circumstances. Clearly, at the one extreme, it would be logical to accept a relatively high risk from the consumption of contaminated food and water supplies during nuclear warfare if such supplies were the only ones available. If, on the other hand, effective, feasible, and relatively non-disruptive protective measures can be instituted, it would make sense to do so at a level of contamination representing a much lower level of risk. In any case, careful evaluation of the impact of available protective measures is an essential part of the development of guidance for handling contaminating episodes.

The National Advisory Council on Radiation, in its report of 1962 to the Surgeon General, Public Health Service, indicated the requirements which a countermeasure against radioactive fallout must fulfill in order to be useful in public health:

"First, it must be effective: that is, it must substantially reduce population exposures below those which would prevail if the countermeasure were not used. Second, it must be safe; i.e., the health risks associated with its use must be considerably less than those of the contaminant at the level at which the countermeasure is applied. Third, it must be practical. The logistics of its application must be well worked out; its costs must be reasonable; and all legal problems associated with its use must be resolved. Next, responsibility and authority for its application must be well identified. There must be no indecision due to jurisdictional misunderstandings between health and other agencies concerned with radiation control. Finally, careful attention must be given to such additional considerations as its impact on the public, industry, agriculture, and government."
Any consideration at the national level of the practical aspects of a currently available protective measure against radiiodine must take into account the resources available for local implementation. By the same token policy decisions that measures are not needed in a given community, state or group of states requires effective resources for public interpretation and official decision-making at local and state levels.

The legal and professional responsibilities of local and State Health Departments, the extent of their resources of laboratories and trained personnel, and their role in matters affecting health are factors which should be taken into account by all who are concerned at the national level with the practical aspects of environmental radiation protection criteria and protective measures.

In view of the potential need for radiation control measures applicable beyond normal source control, many State health agencies have felt it desirable to develop operational plans for such measures to satisfy both public health and public relations responsibilities.

Dr. Clarkson's statement included the following:

U.S. Department of Agriculture research on countermeasures against radioactive fallout includes an evaluation of various phases of fallout from its deposition on crops and soils and the protection of crops and feeds from fallout to the removal of the radionuclides from the food itself.

Countermeasures are designed to minimize the intake of radionuclides by farm animals and develop farming practices that will reduce the availability of radioactive materials in crops. The objective of all studies and measures is to minimize the consumer's intake of fallout radionuclides in foods. Three major areas of research have been conducted by the Department toward the development of countermeasures that might be used to reduce radioactive materials in agricultural commodities. These are (1) the removal of radioactivity in milk; (2) animal management; and (3) soil treatment to reduce radioactive material in crops. The following will provide further details on each of these areas of research.

Removal of Radioactivity from Milk

Research on removal of fallout radionuclides from milk has been pursued in a project at the Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, and the Robert A. Taft Sanitary Engineering Center, Public Health Service, Department of Health, Education and Welfare, Cincinnati, Ohio.

This project has been jointly and equally supported for the past three years by the Departments of Agriculture and Health, Education and Welfare, and the Atomic Energy Commission. It has been carried out in the two laboratories under the direction of a Joint Advisory Committee of the three agencies.

The primary objective has been to develop a standby process for use in an emergency to remove strontium 90, with lesser attention to other radionuclides. It was considered to be of major importance that the milk
be changed as little as possible in nutritional value and flavor, and that the process developed be one that could be carried out in an operating dairy at reasonable cost. These objectives have been achieved on a pilot-plant scale.

Over 90 percent of the strontium 90 can be removed by ion-exchange resins without significant effect on the nutritional value and flavor of the milk. Barium 140 and cesium 137 are also removed. Preliminary cost estimates based on these pilot-plant studies are 0.9 cents per quart if operated on a full commercial-scale of 100,000 pounds of milk a day, or more.

Full commercial-scale trials to establish feasibility of the process and secure more reliable cost estimates are needed. The U. S. Department of Agriculture received $100,000 in fiscal year 1963 earmarked for this purpose. The Department of Health, Education and Welfare is contributing an amount equal to that supplied by this Department because of the high priority of this work. A research contract is being negotiated with the Producers Creamery Company, Springfield, Missouri, to carry out this commercial-scale feasibility test. The experimental work will be done at their Lebanon, Missouri, plant.

In the same project, the removal of iodine 131 by means of an ion-exchange process is being developed on an automated pilot-plant scale at the Agricultural Research Center, Beltsville, Maryland. The process appears to be feasible at this stage of study.

Animal Management

IODINE 131

Some short-range studies to determine the usefulness of various feeding-management systems, with dairy cows in areas of high incidence of fallout, in reducing the iodine 131 content of milk have recently been concluded. The studies were conducted over a two-month period in the fall of 1962 by the Agricultural Research Service, U. S. Department of Agriculture, in cooperation with the Public Health Service, Department of Health, Education and Welfare, and the Agricultural Experiment Stations of Minnesota, Iowa, and Utah.

The four feeding-management systems studied included:

(1) all roughage from pasture, with no access to shelter;
(2) 50 percent of roughage from pasture and 50 percent from stored feed, with access to shelter;
(3) all roughage from stored feeds, with access to both dry lot areas and shelter; and
(4) all roughage from stored feed and under shelter at all times.

Five cows were placed in each treatment group at each location. All of the stored feed used had been in storage for 45 days or longer.

The more important conclusions that can be drawn from the results are:
(1) removal of cows from pasture and feeding stored feed is a very effective means of reducing iodine 131 levels of milk;
(2) at the levels encountered in this study, inhalation and direct contact are insignificant sources of iodine 131 in milk;
(3) Iodine 131 in milk is reduced to insignificant levels within three or four days after the cows are removed from a contaminated pasture; and
(4) iodine 131 in milk approaches its maximum level within three or four days after cows are placed on a contaminated pasture.

It can be concluded that milk can be produced with insignificant levels of iodine 131 contamination if the cows are prevented from obtaining it in their feed. The most likely cause of feed contamination is pasturage. Stored food should be in storage at least 45 days to allow for deterioration of iodine 131 contamination.

In periods of significant fallout of iodine 131 from the atmosphere, the contamination of milk by this radionuclide can be avoided or reduced to very low levels in a short time by changing lactating cows from a pasture-feeding system to one of feeding 45-day old stored feeds.

**STRONTIUM 90**

Laboratory experiments have shown that strontium is metabolized by cows and other animals in a fixed relationship to the metabolism of calcium. The variations in these relationships have been small enough under the limited conditions of laboratory experiments that it appears possible to predict the level of strontium 89 & 90 contamination of milk in the field by knowing the levels of strontium and calcium in the feed. If the comparability of laboratory results and field trials is real, recommendations for reducing strontium contamination of milk through alteration of calcium levels, hay-grain ratios, etc., can be made directly from laboratory findings.

Existing data relating field observations on strontium to laboratory results is variable and inconclusive. There is reason to believe, however, that a well-controlled field test of laboratory developed concepts of the metabolism of strontium and calcium would confirm predictions from laboratory results and make unnecessary the conduct of field trials related to recommendations for reducing strontium contamination.

The main objective of field trial research presently being initiated is to determine if the effect of altering calcium-strontium ratios in feeds under field conditions is similar to that which can be predicted from laboratory results. This will also determine under field conditions if alterations of calcium-strontium ratios will reduce the contamination of milk by strontium. Among the factors to be studied are the effect of different feeds, hay-grain ratios, calcium levels, stage of location, and level of production of the cow. The work will be carried out at three locations (Iowa, Minnesota, and Utah) with twelve cows at each location.

Research to date on the effectiveness and selectivity of a chelating agent as a feed ingredient in dairy cattle rations to reduce the uptake and
the secretion into the milk of strontium and other undesirable mineral elements must be classified as strictly exploratory. Following the initial trial on one cow during the summer of 1962, another trial was conducted which confirmed the earlier observation that vermiculite did lower the strontium content of milk. Results also showed some lowering of cesium and barium, but not of iodine 131. It was evident that a conclusive answer would have to await a more extensive study.

The matter has been considered in connection with planning of work in the radioactivity laboratory at the Agricultural Research Center, as well as in discussions with the Utah, Iowa, and Minnesota cooperators.

A rather closely controlled experiment will be needed that checks on just what effect the vermiculite has on metabolism, both desirable and undesirable. However, this study will have to await the availability of funds and laboratory facilities for the purpose.

**Soil Treatment to Reduce Radioactive Material in Crops**

Research studies on soil decontamination have been conducted in co-operation with the Atomic Energy Commission for a number of years. These include the development of effective procedures for the removal of radioactivity from farmland.

Field experiments have shown that when fallout is deposited on lands where standing crops are growing, no more than one-third of the deposit can generally be removed by harvesting the above portion of the plants by mowing.

Decontamination of soil by removing the ground cover is effective when such cover is thick enough. For example, by placing a heavy mulch of five tons of straw per acre on the soil surface prior to fallout, and then raking it after the fallout is down, will remove about 50 percent of the contamination.

The physical removal of surface contamination has been investigated because it seems to offer the highest degree of decontamination among methods that might be applied to soils. However, the method is expensive and at present requires the removal of several inches of soil to attain a high degree of decontamination. Close to 100 percent removal can be obtained by scraping the smooth seedbed with a small road grader.

Cutting and disposal of sod will also provide a means of removing up to 90 percent of the contamination of the surface of the land.

These countermeasures could be used against strontium 90 if it were present in the soil in large amounts constituting a serious hazard to the health of man. It must be emphasized that these countermeasures are extremely drastic and costly; however, they might be necessary to reduce the radioactivity in areas highly contaminated with strontium 90 following a nuclear attack or in small areas seriously contaminated by radioactive materials following a nuclear reactor accident.

Studies in soil management have revealed that the addition of lime, gypsum, fertilizer, or organic matter in practical amounts to low calcium soils will help reduce the uptake of strontium by the growing plants by less
than one-half. For most soils and crops, it is recommended that lime not be applied in excess of the amount of calcium needed for maximum crop growth. The addition of lime to acid soil is included in good farming practices and is applicable as a countermeasure at any time. However, in heavily contaminated acid soil or in soil needing no calcium, this method would not be effective.

Other Areas of Research

REMOVAL OF STRONTIUM 90 FROM WHEAT

Research on improving the removal of strontium 90 from wheat by alterations in the milling process and new treatments of the wheat berry prior to milling is being initiated by the Agricultural Research Service in fiscal year 1964. Advantage will be taken of the excellent milling facilities and know-how of the Northern Utilization Research and Development Division, Agricultural Research Service, Peoria, Illinois.

These studies are planned to be conducted cooperatively with the Health and Safety Laboratory of the Atomic Energy Commission in New York which has carried out monitoring of wheat for strontium 90 for some years and has made exploratory studies of this problem.

FRUITS AND VEGETABLES

Research is also being planned by the Agricultural Research Service on removal of fallout radionuclides from fruits and vegetables to be initiated in fiscal year 1964.

Studies will be conducted to determine the extent to which purely external fallout can be removed from representative vegetables and fruits by the normal processing operations used in the food industry. Suitable commodities might include peas, spinach, tomatoes, apples, oranges, blackberries, and cherries.

Another phase of the research will be to determine in some detail the distribution of strontium within the edible parts of a wide variety of vegetables and fruits that are commercially processed. This will reveal the distribution pattern to be expected when strontium 89 and strontium 90 are absorbed through the roots (internal contamination).

The U.S. Department of Agriculture will continue to conduct research in its quest for new and more effective means of reducing and minimizing the amount of radioactive material in agricultural commodities that can be placed on a standby basis to be used if such is ever required. It will continue to work cooperatively with those agencies having related interests and responsibilities.4

The Public Health Committee wishes to commend Drs. Chadwick and Clarkson for this excellent report.
REFERENCES

4. Excerpts from statements before Subcommittee on Research, Development and Radiation, Joint Committee on Atomic Energy, made by Dr. Donald Chadwick, of U. S. Public Health Service, and Dr. M. R. Clarkson, of Agricultural Research Service, and Dr. M. R. Clarkson, of Agricultural Research Service; Frank A. Todd, ARS, USDA, Washington, D. C.
SCRAPIE IN THE UNITED STATES DURING THE 1963 FISCAL YEAR (A Situation Report)

J. L. Hourrigan, D.V.M.

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During fiscal year 1963, there were eleven scrapie-infected flocks reported in six states. The infected flocks, source flocks, exposed sheep, and their immediate progeny slaughtered as a result of these outbreaks involved the payment of Federal indemnity on 142 claims for 5,068 sheep slaughtered from eleven states. Federal indemnity of $120,596 and state indemnity of $36,583 was paid. There are presently some 638 flocks of approximately 231,829 sheep in 26 states under surveillance for scrapie. This is a decrease from the previous year when 1,236 flocks were under surveillance.

Illinois. There were four scrapie outbreaks reported during fiscal year 1963 bringing the state total to 28—more than any other state. Infected flock Illinois No. 25 was reported by the owner. Suffolk ewe No. 491 had been purchased from another flock (Flock No. 27) and was showing signs of scrapie 36 months later. The flock of 49 sheep in LaSalle County and approximately 532 exposed sheep or their immediate progeny in some 20 Illinois flocks were slaughtered. No interstate movements were involved.

The outbreak in flock No. 26 containing 58 sheep in Champagne County was found on routine inspection. The infected Suffolk ewe was not registered. She had been purchased 18 months earlier along with the entire "source flock." No additional sheep were considered exposed.

The infected Suffolk ewe in flock No. 27 was reported by the owner. The large De Kalb County flock of some 1,400 sheep had included those from a number of possible sources of the disease; however, infected ewe No. 122N had been imported from Canada 31 months prior to showing signs of scrapie. The flock included sheep of the Suffolk, Hampshire, Cheviot, and Cotswold breeds. The outbreak occurred just prior to the end of the fiscal year and slaughter was pending on June 30.

Infected flock No. 28 was reported by a veterinary practitioner. Suffolk ewe No. 230 had been bred in a Tazewell County flock. The flock of 179 sheep was slaughtered as were exposed sheep and their immediate progeny.

Virginia. Three infected flocks were reported during the fiscal year. All infected sheep were Suffolks. Infected flock No. 5, in Augusta County, was reported by a veterinary practitioner. Ram No. A590 was showing signs of scrapie 24 months after entering the flock of 152 sheep. There were no additional exposed sheep to be slaughtered.

Infected flock No. 6, in Rockingham County, was reported by the owner who suspected sheep scabies. Ram No. 79E had been bred in Virginia...
infected flock No. 2 found to have scrapie in June 1961. The Rockingham County flock contained 75 sheep. There were no additional exposed sheep identified as subject to slaughter.

Infected flock No. 7, in Albemarle County, was reported by a county agent as possibly having scabies. Infected ram No. 27H had also been bred in flock No. 2 and was showing signs of scrapie 30 months after entering the flock of 169 sheep. There were no additional exposed sheep to be slaughtered.

Texas. Infected Suffolk ewe No. 27 in Texas infected flock No. 3 had been bred by the Hamilton County owner. The disease was reported by the owner who felt that he had seen other sheep with the disease in his flock of 48 sheep. Ewe No. 301, previously sold from the flock, was found to have scrapie in infected flock No. 4 (to be reported in fiscal year 1964). Recommended eradication procedures are being followed.

Iowa. Iowa's second infected flock was reported by a veterinarian. Suffolk ram No. 48-59 was showing signs of scrapie 21 months after entering the Carroll County flock of 75 sheep. The infected flock and approximately 70 exposed sheep in eight Iowa flocks were slaughtered.

Missouri. Infected flock No. 3 was reported by a veterinarian. Suffolk ewe No. 57 was bred in the Clay County flock of 125 sheep. Exposed sheep and their immediate progeny slaughtered included approximately 413 sheep in 32 Missouri flocks, 30 in nine Kansas flocks, and some 173 in eight Texas flocks.

West Virginia. The second infected flock in the state was reported by a veterinarian. Suffolk ram No. 13 had been purchased from another West Virginia flock and was showing signs of the disease 27 months after entering the Marion County flock of 22 sheep. Recommended eradication procedures were followed.

SCRAPIE RESEARCH DEVELOPMENTS

Your Committee's report for the past two years included summaries of the considerable work and progress being made in the field of scrapie research. This work is continuing and the present report will merely cover some of the highlights of current developments.

W. S. Gordon, Director of the British Agricultural Research Council's Institute for Research on Animal Diseases wrote furnishing current information in further regard to his report before this organization at our San Francisco meeting. At that time, he reported a wide range of susceptibility in groups of sheep drawn from 24 British breeds, the most susceptible being the Herdwick and the most resistant, the Dorset Down. They now have at Campton approximately 1,000 ewes in 50 groups, each with a ram, and representing both breeds. These have produced a first crop of lambs which are under test at present. There have been some 130 cases of scrapie in the Herdwick lambs and no cases in the Dorset Downs so far, although all were inoculated at the same time with the same scrapie material. The breeding groups are in isolation and have now produced a second crop of lambs which will remain in isolation when weaned and the
parents will be removed and tested for susceptibility. As a result of these tests, both of parents and first crop lambs, the second crop lambs remaining in isolation will be classified for susceptibility according to the susceptibility shown by their fathers, mothers, sibs, and half-sibs. With animals of known susceptibility, he hopes to be able to settle a number of the problematical questions about scrapie that still require an answer.

At the Institute, they have transmitted scrapie successfully to mice both with the "sleepy" goat strain of scrapie and with brain material from a scrapie-infected sheep. The agent has now been passed successfully in mice and this has been confirmed by Stamp and colleagues at Moredun, by Gujdusek at Bethesda and by Eklund and Hadlow at the Rocky Mountain Laboratory.

The Institute's biochemist, G. D. Hunter, has completed experiments which suggest the agent is particulate and approximately 50 μ to one μ in size. He also has some evidence to suggest that an RNA preparation was infective for mice.

The Institute's virologist, D. A. Haig, has some interesting results in tissue culture, both in embryonic goat cells and in embryonic mouse cells. He has demonstrated pleomorphic particles both intra-cytoplasmic and extracellularly in these cultures but advises that it is too early to say whether or not this is the transmissible agent of scrapie. Animals inoculated with these cultures are under observation at present. One goat has died with lesions indistinguishable from scrapie and three mice have also shown similar lesions. Time and much more work will tell whether this result is significant and if it can be confirmed.

We are much in debt to Dr. Gordon for this current information, much of which is not yet published.

J. Anthony Morris (Division of Biologics Standards) and D. Carleton Gajdusek (Project for the Study of Slow, Latent, and Temperate Virus Infections), National Institutes of Health, Bethesda, Maryland, published on their work with mice inoculated with scrapie sheep or scrapie goat material. Their paper appears in "Nature," Volume 197, No. 4872, pages 1084-1086, March 16, 1963.

The scrapie-like disease appeared two and one half to 10 months following inoculation in the case of the sheep material and 6-17 months following intraperitoneal or intracerebral inoculation of infected goat material.

The authors report that breeding experiments to determine whether the disease in mice can be genetically or congenitally transmitted are in progress. They are also performing experiments to determine whether neutralizing antibody develops in affected sheep or mice and to investigate whether immune tolerance may exist to the scrapie agent inoculated in utero or early in neonatal life, as is the case in lymphocytic choriomeningitis in mice with subsequent persistent infection.

C. M. Eklund, W. J. Hadlow, and R. C. Kennedy (U.S. Department of HEW, PHS, NIH, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana) presented a paper SOME PROPERTIES OF THE SCRAPIE AGENT AND ITS BEHAVIOR IN
The authors reported that a disease syndrome was seen in mice following intraperitoneal, intranasal, and subcutaneous injections of infected mouse brain suspensions. The disease was also seen in litters born to female mice inoculated intracerebrally. Significant morphologic changes were observed in the central nervous system.

The agent was demonstrated by inoculation to be present in the brain, spleen, thymus, and liver of infected mice. The size of the agent appeared to be on the order of 50 μ.

The preliminary findings suggest that the scrapie agent is not a member of a unique group of pathogens but is a medium-sized virus whose resistance to heat is analogous to that of serum hepatitis virus. However, the prolonged incubation period, the protracted course of the disease, and the absence of inflammatory changes in brain and spinal cord comprise a pathologic entity hitherto unknown among viral infections of the central nervous system.


The authors concluded that direct inoculation into mice of brain suspensions from a Suffolk sheep experimentally infected with scrapie and from goats affected with the scratching syndrome produced a disease with a rapid course following an incubation period of seven and 12 months respectively.

Subinoculation of brain and spleen suspensions from subclinical cases of scrapie in mice fed either brain or spleen from Suffolk sheep affected with natural scrapie resulted in a fatal clinical disease in mice.

The scrapie agent from both sheep and goats was maintained through three passages in mice. In the third passage the disease appeared to have a very rapid course after an incubation period of less than five months. A progressive shortening of the incubation period was obtained in succeeding passages.

The course of the disease in mice may be very rapid, as in the hyperexcitable type of disease, or rather protracted. The most common form of scrapie in mice is the lethargic type, the clinical course of which is variable, some mice dying within one to three days while others survived for one to three weeks.

The pathological changes after inoculation of tissues from scrapied Suffolk sheep and goats affected with the scratching type are confined to the brain. Neuronal vacuolation is invariably present in subcortical centers of the pons and mesencephalon where the vestibular nuclei as a rule are always affected. Status spongiosus of the grey matter is commonly present in the anterior parts of the brain and, in addition, to the thalamus and corpus striatum, the cerebral cortex and hippocampus are also affected.
Oedema of the white matter is also seen in some brains and the white matter of the cerebellum is more often affected by oedema than any other part of the brain.

Scrapie in mice has been transmitted by the intracerebral, intragastric, and intraperitoneal routes. The incubation period is longer after intraperitoneal than after intracerebral inoculation.


The authors summarized as follows: "Homogenized brain, extracts, and residue of brain from normal and scrapie sheep were inoculated into 116 sheep. Of 72 sheep inoculated with scrapie material, 27 developed the disease; whereas, four of the 44 inoculated with 'normal' brain material showed symptoms similar to those of scrapie. The scrapie agent survived extraction for 18 hours with diethyl ether followed by water for 24 hours. Similarly, the agent survived extraction in a Soxhlet apparatus with 95 percent ethanol containing 2.0 percent 4 M HCL for 24 hours.

There was no evidence of demyelination in sections of brain and spinal cord supporting the view that allergic encephalomyelitis is not responsible for the scrapie symptoms. Liver function, as measured by the bromsulfalein test, remained normal until just before death. These results preclude that liver dysfunction contributes to the disease but do not exclude the possibilities of other metabolic derangements."

(Submitted by J. L. Hourrigan, D.V.M., U.S.D.A., A.R.S., Hyattsville, Maryland.)
CONTAGIOUS ECTHYMA IN SHEEP AND GOATS
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Contagious ecthyma, commonly called soremouth in most of the sheep-raising areas of the West and Southwest, is a disease of sheep and goats.

The signs of soremouth include slight swelling of the lips, inflammation and papule formation followed by pustules which rupture and coalesce, forming a thick, brownish scab which adheres tightly for approximately three weeks.

The disease has been recognized by sheep and goat raisers for many years, but, due to the low mortality rate, it was not considered of particular importance until it was found to occur on approximately 80 percent of the ranches in West Texas. On some ranches, 100 percent of the lambs and kids were affected. Because of the increase in mortality resulting from the tremendous build-up of screw worm cases, a study was initiated. If death did not occur from myiasis, the loss in flesh and cost of treating infected lambs and kids made sheep and goat production unprofitable on many West Texas ranches.

The initial work on soremouth was started at the Ranch Experiment Station in 1928 by Dr. E. A. Tunnicliff, assisted by the late Dr. H. Schmidt, Research Veterinarian at the Ranch Experiment Station, and Head of Veterinary Research, Texas Agricultural Experiment Station, College Station, Texas, respectively. The objectives of the project were to determine the cause of the disease and a practical remedy or means of control.

A review of the early literature revealed that several research workers had described diseases known as necrobacillosis and lip-and leg ulceration attributed to necrobacillosis. This resembled to some extent the disease known as soremouth, with the exception that the lesions on the feet and legs were never recorded by Texas research workers. Research workers in Indo-China and South Africa observed lesions on both sheep and goats similar to those seen in Texas. They termed the disease contagious ecthyma.

The disease was readily transmitted from infected animals to susceptible animals by scarifying the lips and using emulsified scabs as the inoculum. The early workers noted that either a natural attack of contagious ecthyma or one resulting from experimental inoculation rendered the animal resistant to further attacks or inoculation.

Glover in 1928 reported that the virus of contagious ecthyma was filtrable. He also reported that only by applying the virus to scarified skin was he able to transmit the disease, and that attempts to infect laboratory animals and fowls were negative. An emulsion of dried scab lesions

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inoculated on the scarified skin of the flank did not produce generalized lesions but did produce satisfactory immunity in the inoculated animal.

THE DISEASE AS IT OCCURS NATURALLY

Soremoutth as it occurs naturally on the range usually affects only young lambs and kids. However, it may occur in animals of any age. Most young animals will have soremouht by weaning time, which is usually in September of the year in which they are born. If a lamb or kid does not contract the disease, it may become infected at any age, even as a mature animal, if it comes in contact with the virus.

Usually the ranchman does not notice the first stage of soremoutth. Only after the large scab has formed or the animal has an infestation of screw worm larvae, does he become aware that his flock has this disease.

In the past, infected animals were treated in various ways, many of which only aggravated the condition and increased screw worm infestation. In one instance, 250 cases of screw worms were noted in a flock of 600 lambs. The ranchman used a burlap sack, which caused bleeding, to remove the scabs, then applied used crankcase oil on the mouths and turned the lambs back to pasture. Five days later, 250 lambs had screw worm larvae in their mouths.

DEVELOPMENT OF SOREMOUTH

The development of soremoutth may be studied by taking a dry scab from an infected animal, drying it and then grinding with mortar and pestle. The resulting powder is suspended in normal saline and applied to the scarified skin of a susceptible lamb or kid, in the flank area, which is devoid of Wool or mohair. On the second day, slight inflammation may be observed along the lines of scarification and on the third day small vesicles develop. On the fourth day there will be slight pustule formations which rupture and coalesce on the fifth and sixth days. On the seventh and eighth days a moist, flexible scab is formed and at this time the disease is evident even to the inexperienced. The scab gradually increases in size and becomes firm, usually reaching its maximum size on about the twelfth to fourteenth days. It then becomes dry and hardens, with healing from the underside, and falls from the animal in approximately 24 or 25 days. A month after healing there is no evidence of a scar even in the most severe cases.

TRANSMISSION

Transmission of the disease may be accomplished readily by experimenental inoculation. It has also been produced by scarifying the lips and rubbing on dirt from the floor of a stable which had previously housed soremoutth-infected sheep. As many of the browse plants in West Texas have thorns and prickly leaves, it is assumed that the lips of lambs and kids are almost constantly abraded and ready for natural inoculation from
the virus that may be on the ground or from the vegetation as a result of scabs that have dropped from healing cases.

Never have we been able to transmit the disease to other farm animals, including hogs, cattle, horses, dogs, guinea pigs, rabbits and chickens. However, the author and four other workers at the Station have developed lesions of the disease. One worker took material from a human lesion believed to be contagious ecthyma and reproduced lesions in a susceptible lamb identical to those in known cases of soremouth.

In work done by Schmidt and Hardy, strains of virus obtained from sheep produced lesions and satisfactory immunity when used on goats. Virus obtained from goats and used to inoculate sheep seemed to produce milder lesions that healed from three to five days earlier, yet produced satisfactory immunity when challenged by either strain of virus obtainable at that time.

The Texas Agricultural Experiment Station, Substation No. 14, located near Sonora, Texas, developed a soremouth vaccine made from the powdered scabs using 1:100 suspension in glycerine and normal saline solution as the vehicle. After two years of experimental vaccination on five ranches, with 7884 vaccinated animals and 10,173 controls, results showed 99.62 percent protection in vaccinated lambs and 65.3 percent infection of unvaccinated lambs. The sheep and goat raisers urgently requested that the vaccine be made available to them. Thus the first soremouth vaccine was made and distributed from Substation No. 14 in the spring of 1933. Since that time, it is estimated that use of the vaccine in Texas alone has resulted in a monetary saving of $750,000.00 per year to the sheep and goat raisers.

Since 1933, different ranchmen have noted that even though good "takes" were obtained from the station vaccine, their lambs would develop soremouth lesions three to four months following vaccination. In these instances, scabs were procured and cross immunity tests were made with animals which had been used to produce scab for vaccine. By this method six variants or different immunological strains were found. All variants have been added to the Station vaccine.

In September, 1961, some scabs were received from the United States Sheep Station at Dubois, Idaho. Animals inoculated with this virus produced lesions extending onto the mucous membranes of the buccal cavity, similar to those produced by ulcerative dermatosis of sheep, and unlike any previously seen in Texas lambs. There were no lesions in the interdigital region. Cross immunologic studies with contagious ecthyma, Texas strains, by skin inoculation gave very poor response for protection. Immunologic studies with contagious ecthyma and ulcerative dermatosis as reported by Trueblood, et al. apparently substantiate our work.

Attempts to grow contagious ecthyma virus on artificial media have been negative. Adaptation of the virus to chick embryos also has been unsuccessful. Livingston was able to adapt the contagious ecthyma virus to tissue culture media of trypsanized lamb kidney cells. Lambs inoculated with the virus after five culture passages produced typical lesions of the disease and were later immune to future inoculations from the same strain.
of virus. However, at this early writing one animal which proved to be immune to the same strain developed typical lesions of soremouth when inoculated with an unrelated strain. This unrelated strain had just been received from an outbreak of soremouth in a brand of lambs that had been vaccinated approximately 45 days previously and had been checked for "takes" about ten days after first vaccination in sufficient numbers to assure close to 100 percent "takes."

**IMMUNITY TESTS**

After observations on many West Texas ranches, and consultation with ranchers, it was determined that soremouth primarily was a disease of young animals. The lambs and kids were in very close contact with their mothers, yet only rarely was a case of soremouth noted in the adult animals. Therefore, it was suspected that there must be an immunity which was developed in the young animal from an attack of the disease and carried over into maturity. With this in mind, a number of lambs were inoculated with soremouth virus. After they were healed, they were held for immunity tests or challenge inoculations. The challenge inoculations were carried out at intervals of approximately 45 days for 18 months. At each challenge the animals proved immune to the disease.

The immunity developed following a natural course of the disease produces a very strong life-long resistance to subsequent attacks from the same strain of virus.

Immunity developed by artificial inoculation or vaccination gives the same results as that produced by natural infection.

Grown sheep from two to six years of age, known to have been vaccinated, have been challenged with the same strain of virus as that used for vaccination when they were baby lambs. In no case have the challenge inoculations produced the large, exuberant scab formation typical of the primary disease. In approximately 25 percent of the animals so challenged the disease shows only a slight inflammation with very few pustule formations which are completely healed within a ten-day period from date of inoculation. Control animals inoculated at the same time with the same virus have shown severe lesions and large scab formations requiring 24 days or more for complete healing.

Sheep which have been immunized as suckling lambs by vaccination with the vaccine strain of virus, when challenged by a different strain of the virus or variant will develop typical lesions of the disease. As a rule, however, such lesions are not as severe as those that develop on a susceptible lamb inoculated at the same time with the same virus strain.

As a pilot for establishing a variant strain of contagious ecthyma virus, we use two lambs that have been inoculated with the known strain of virus and recovered; two lambs that have been inoculated with the unknown strain of virus and recovered, and two susceptible lambs. By using a cross inoculation with the two strains on the immune lambs and checking on the susceptible lambs, we are able to pick up the variant strain of virus for further study.
There is no immunity to the disease passed from the immune ewe to her lamb, nor does the colostrum from an immune ewe contain any perceptible protective substance.

VACCINATION

Inoculation of the virus into very young lambs and kids produced an immunity of considerable duration, and experimental field vaccinations had proven successful. It therefore was decided to make a vaccine for sore-mouth available to the sheep and goat producers of Texas.

The first vaccine was distributed from the Texas Agricultural Experiment Station located near Sonora, Texas in the spring of 1933. Since that time the Station, as the insistence of the Texas Sheep and Goat Raisers Association, has made the vaccine available to the producers either direct or through livestock medicine dealers, wool warehouses and veterinarians.

The vaccine produced by the Station is for sale in Texas only, as no interstate license has been procured.

The vaccine is constantly checked for potency and new variant viruses are added to give maximum protection.

It is sent out in two forms: 1) as a suspension of the live scab virus 1:100 dilution in 30 percent glycerine and 70 percent normal saline. This type of vaccine carries an expiration date of about 50 days. 2) The second type of vaccine is in two small vials—one containing the live scab virus powdered and, one containing a sterile diluent consisting of 30 percent glycerine and 70 percent saline. This carries an expiration date of six months. Both types of vaccine are put up in 100 and 250 dose sizes. The 100-dose vial is by far the most popular. It carries 60 mgs. of live scab virus powder and six cc. of diluent.

Approximately one million doses per year are made and distributed by the Station and it is estimated that three million lambs and kids are immunized annually. Very few ranchers vaccinate less than 200 lambs from each 100-dose bottle, and in some instances it has been reported that 1000 have been vaccinated from one 100-dose bottle.

The most common site for inoculation is the inner aspect of the rear thigh where the skin is devoid of wool or mohair. One or two small scratches 1/4 inch apart and 1/2 inch long and a small amount of vaccine applied to the scratches provides satisfactory results. Some ranchers have dipped an ice pick into the bottle of vaccine and used it to pierce the ear, a method not approved of at this Station. However, it seems to have given good results in many cases, and is not only convenient to the vaccinator but allows him to inoculate more than 100 head per 100-dose bottle.

LONGEVITY OF THE VIRUS

At an early date in the investigations, a study was undertaken to determine how long the virus would remain alive in both the dry form and the suspension. The dry virus protected from the sun but subjected to fluctuating temperatures survived over three years, or until the sample was...
depleted. The suspension survived at room temperatures for a little over a year. Virus in a dry, powdered state in a sealed amber bottle, under refrigeration at approximately 45°F was still viable after 22 years and eight months. Virus from this lot tested during the summer of 1963, after 27 years, three months and 11 days storage, produced severe soremouth lesions on susceptible lambs two months of age when the lips were scarified and a 1:100 suspension was rubbed into the scarifications. Some of this virus remains in the ice box to be tested at a future date.

**TREATMENT**

Medicinal treatment of soremouth after lesions are observed is ineffective. The most practical treatment is use of some bland oil to keep the scabe soft and pliable, and possibly a disinfectant to decrease the infection. Vaccination of all lambs is recommended if an outbreak is observed in a flock. This will start the immunization process in those animals not infected and shorten the duration of the disease in those already showing lesions.

**SUMMARY**

Contagious ecthyma (soremouth) of sheep and goats at one time was very prevalent in the sheep and goat raising sections of Texas, and its severity threatened the economy of lamb and kid production in the area. Early studies suggested the practicability of producing a vaccine to control the disease. Experimental work proved that immunity developed from an artificial inoculation was solid and of long duration and that a vaccine could be made that would produce satisfactory protection in range sheep.
The vaccine produced contains several variants of the virus. It does not afford satisfactory protection from the virus of ulcerative dermatosis (lip-and-leg ulceration).

REFERENCES

OVINE VIBRIOSIS
(A Situation Report)
B. D. Firehammer, M.S.
Bozeman, Montana

Research has shown that excellent results can be obtained through the use of formalin-killed *Vibrio fetus* vaccine used prior to breeding or early in pregnancy. Only fair to poor results can be expected if the vaccine is used in the face of an outbreak. At the present time, vaccine of serotype I (Marsh and Firehammer typing) is the only type available from commercial firms. Although outbreaks due to serotype V are very rare in Colorado and Idaho, they are common in Montana and possibly in some other states. The two serotypes do not produce cross-immunity, but research has shown that a bivalent, mineral oil-adjuvant vaccine is feasible and it is hoped that it will soon be available.

Field trials with antiserum have not proven encouraging. The feeding of antibiotics to stop losses after an outbreak has started does not appear to be effective, although their use prior to an outbreak is of benefit as a preventative.

Present recommendations for the control of vibriosis include vaccination of all replacement ewes each year and where possible, the replacements should be obtained from the sheepman's own herd. Sheep which have been involved in an outbreak are considered immune to re-infection with the same serotype. However, it is felt that a small percentage of such animals may be carrying the organism, possibly in the intestinal tract or bile, thereby constituting a hazard to susceptible pregnant ewes. There is some reason to believe magpies may harbor the organism in their intestinal tract, and that feed and water contaminated with their droppings is a source of ewe infection.

Research is being continued to determine the duration of immunity obtained with adjuvant vaccines, to improve methods of identification and growth of the causative organism, and to learn more about the manner in which the disease is spread. (Submitted by B. D. Firehammer, M.S., Associate Bacteriologist, Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, Montana).
ENZOOTIC OVINE PNEUMONIA
(A Situation Report)
D. L. Dungworth, D.V.M.
Davis, California

The acute form of enzootic pneumonia ("Shipping fever" or "Summer pneumonia") is responsible for severe sheep losses, usually following some type of environmental stress. A sub-clinical form, recognized by the presence of sub-acute to chronic lesions in the lungs of a large portion of slaughtered sheep, also occurs. These lesions are important because they act as a nidus for the flare-up of an acute pneumonia and possibly, if severe enough, cause reduction in feed conversion and retardation of weight gain in feeder lambs. However, there are no experimental data to support this latter possibility.

ETIOLOGY

Most investigations have dealt with the role of microorganisms but some researchers have attempted to include the role of environmental stressors in their experimental methods also.

1) Viruses. The only virus of established importance is a member of the psittacosis-lymphogranuloma venereum (PLV group). This has been shown experimentally to produce an extensive but clinically mild pneumonia which soon resolves. The lesion resembles many of those found in the lungs of slaughtered lambs. It is not possible so far to estimate the degree of importance of the PLV virus because of difficulties encountered in its isolation from old lesions and the absence of pathognomonic features of the pneumonia which it produces. Undoubtedly there are other agents, or combinations of agents, which can produce a similar effect.

The search for other viruses present in either pneumonic lungs or normal respiratory tracts of sheep has lagged considerably behind corresponding work in cattle. The only reports in recent literature are a preliminary one from New Zealand mentioning six isolants of unidentified cytopathic agents from cases of pneumonia and one from Hungary describing the isolation of an influenza strain. Myxovirus parainfluenza 3 viruses are widely distributed in cattle and although by themselves produce clinically mild or inapparent infection, appear to play some part in the etiologic complex of shipping fever. Reoviruses and adenoviruses have also been isolated from cattle but their pathologic importance has not been established. It would be surprising if a similar spectrum of viruses were not present in sheep, particularly where management practices lead to crowding. Even after their isolation it is to be expected that evaluation of their importance will be difficult.

*These agents are included here for convenience even though it is realized that many authorities do not include them with true viruses.
2) **Bacteria.** The exudative nature of the acute pneumonia indicates a bacterial lesion. This, together with the high frequency of isolation of *Pasteurella* particularly *P. hemolytica*, leaves little doubt that these bacteria play the most important part in the ultimate production of the acute disease. Attempts to reproduce pneumonia by *Pasteurella* alone, have been inconclusive.

Important developments in the study of *P. hemolytica* associated with sheep diseases have been reported in recent years. On the basis of cultural characteristics, Smith has divided the strains into Types A and T,\(^{21,22}\) the former being recovered from septicemia in young lambs and from enzootic pneumonia, the latter from septicemia in lambs six to seven months old. Strains of *P. hemolytica* from a variety of animals have been divided by Biberstein *et al.* into 11 serotypes.\(^{2,3}\) The majority of the strains were isolated from sheep at least four different important serotypes were associated with pneumonia. This fact will be of special significance when considering prevention. A correlation has also been made between serotypes and the A and T grouping.\(^4\)

Other bacteria, such as *Corynebacterium pyogenes*, seem to be of much less importance. They are of more significance when a frank suppurative process supervenes.

3) **Mycoplasma spp.** (PPLO). These organisms can be isolated both from the upper respiratory tract of normal sheep and from pneumonia lungs.\(^7,4,9\) They have not been shown to be of major importance in the production of pneumonia but might act as a contributory predisposing factor.\(^8\)

4) **Stress.** Although some form of environmental stress is of importance in triggering acute enzootic pneumonia, as shown by the epidemiology of the disease and by failure to reproduce it experimentally with consistency in the absence of stress factors, little is known of the mechanisms whereby it affects resistance and homeostasis of the lung. The critical level for inception of the pneumonic process is in the region of the terminal and respiratory bronchioles. Here there is the interplay between factors of host resistance, bacterial virulence and homoestatic lung mechanisms. Possible ways in which stress might act are in causing the release of adrenocorticosteroids, which reduce non-specific host resistance, and by changes such as chilling and dehydration interfering with function of the mucociliary blanket in the respiratory tract.\(^25,2\) Whatever the basic mechanisms, it is the failure to reproduce adequate stress situations experimentally which makes it difficult to demonstrate unequivocally the importance of organisms such as *Pasteurella*, in the sense of fulfilling all Koch's postulates.

A generalization which appears to hold true is that acute enzootic pneumonia is usually produced by *Pasteurella* spp. alone, when predisposed by sufficient environmental stress, or by these bacteria in combination with pre-existing lesions, concurrent viral infections, or both. The fine points of this statement have still to be solved.
This section must necessarily take the form of suggestions for future developments because there is little of practical significance to report. Prevention of the acute disease outbreaks is the first aim. Since *P. hemolytica* is of predominant importance in this form, it is the obvious organism against which to immunize. Carter lists the two main requirements of an optimum Pasteurella vaccine as:

1) Inclusion of appropriate serotypes
2) Use of virulent capsulated organisms to prepare an oil-adjuvant type vaccine of maximum potency.

Following the establishment of several important serotypes concerned in ovine pneumonia, the production and trial of a potentially useful vaccine is now possible.

The production of efficient anti-viral vaccines appears less promising at the moment because of the probable multiplicity of types involved and the fact that, so far as has been established, viruses usually act as pre-disposers for bacterial infection in causing severe pneumonias. Experience in cattle has been that although a parainfluenza 3 virus vaccine can prevent experimental infection with that virus, it has been unsuccessful in preventing natural outbreaks of shipping fever. However, more study is necessary to amplify this point.

Preliminary work has demonstrated that intratracheal exposure to the PLV virus produces little or no immunity against a second bout of pneumonia. The use of an adjuvant vaccine similar to that used for enzootic abortion in sheep (also a PLV virus) will have to be investigated. Prevention of the subclinical milder lesions of whatever cause is not likely to be particularly fruitful, partly because of the variety of agents which may well cause them and partly because of the nature and dynamics of the lesion. An attempt using a mixed Pasteurella, PPLO virus and PLV virus vaccine has been unsuccessful.

Attempted prevention of the acute disease by reducing as far as possible the number and degree of environmental stressors known to be associated with outbreaks is an obvious complementary approach.

Prevention by feeding broad-spectrum antibiotics to lambs before shipping them to feed-lots has been unsuccessful, but oral treatment with sodium-sulfamethazine some seven to 10 days following transport induced stress has been moderately effective.

REFERENCES

OVINE FOOT ROT  
(A Situation Report)  
A. K. Kuttler, D.V.M.  
Boise, Idaho

**Name of State** | Is foot rot an economical problem in your State? | Do you concur with Nat'l Foot Rot Committee Report? | Is foot rot reportable disease? | Do you issue quarantines? | Have you adequate laws/regulations to eradicate foot rot? | Does industry support eradication? |
--- | --- | --- | --- | --- | --- | --- |
Cal. | Yes | Yes | Yes | No | Yes | Yes, we would if we had a program. |
Ill. | Unknown | Have not received copy | No | No | No | Have not discussed eradication with industry groups. |
Ky. | Not as serious as previously thought | Yes | No | Yes | Yes | Yes |
Maine | No | Yes | Yes | Depends on conditions | Yes | Questionable |
Mich. | Yes | Yes | Yes | No | Yes | Yes |
Mont. | Yes | Yes | Yes | Yes | Yes | Yes |
Ohio | Yes | Yes | No | No | Yes | Yes |
Okla | No | Yes | Yes | Yes | Yes | Yes |
Tenn. | No | Yes | No | No | No | Yes |
Utah | Yes | Yes | Yes | Yes | Yes | Yes |
Vt. | Yes | Yes | Yes | Yes | Yes | Yes |
N. Mex. | No | Yes | No | No | Yes | Would do so. |
N. Y. | No. Sheep industry not extensive in N.Y. Occasional foot rot reported in feed lot lambs, but apparently not serious. |
N. C. | No. Very few sheep in State. |
S.C. | No. We have so few sheep that two flocks account for 75 percent of the total. |
Wash. | No. Foot rot in sheep is a reportable disease in Washington, and quarantines are issued when reported. The number of cases reported in the past few years has been negligible. 

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Wisconsin

Foot rot is a problem in Wisconsin, and is quarantined to the extent that our general statutes prohibit the sale or movement of any animal affected with a contagious or communicable disease. There is presently no program for control or eradication of foot rot in this state, although in cooperation with the University of Wisconsin an industry-wide committee is being appointed to study this problem and see what approach can be made for foot rot eradication.

The following states replied they did not consider foot rot in sheep presented a serious economic problem in their State: Alabama, Arizona, Arkansas, Connecticut, Delaware, Texas, Florida, Kansas, Louisiana, Maryland, Massachusetts, Minnesota, Mississippi, Nevada, New Jersey, North Dakota, Pennsylvania, Rhode Island, South Dakota and Wyoming.

We regret the report is incomplete, and wish to express appreciation to the Livestock Sanitary Officials who did reply to our questionnaire.

Idaho

Foot rot has presented a serious problem in Idaho for many years. I have discussed this disease with many of the large sheep producers, and most of them have had experience with it at one time or another. Unfortunately, many of them confuse foot rot with foot abscess. A common statement from those who have had foot rot in their sheep is that it does as much economic damage, aside from shipping restrictions, and is more difficult to eradicate than sheep scabies.

Most sheep men are of the opinion that foot rot remains in the soil. This is readily understood from the fact that until the horny tissues of the feet are softened as a result of moisture in low wet pastures or in lambing sheds, the disease is somewhat dormant. Thirty days at the most.

I have never talked to a progressive sheep producer who does not agree that if foot rot can be eradicated, it should be done. We do encounter rather serious opposition, as is usually the case, from the individual when his herd is involved. The following letter, which appeared in the June 1963 issue of the National Wool Grower, illustrated this point:

"I hardly know how to start this letter, as I am rather ashamed of the way I acted one year ago in regards to this foot rot problem, as I had no faith whatever in being able to eliminate and cure all foot rot cases and eliminate this disease in one years time.

I have had foot rot in my sheep for over 20 years, and all that time I had run from 5,000 to 6,000 head of sheep. This disease has cost me thousands of dollars, and it sure has been a happy surprise to me to be able to cure all cases and eliminate this disease in one years time.

When you have the right method and plans for control, which Artell Sitter instructed me to use, it really works wonders.

Thanking you for helping me rid my flocks of this dreadful disease, I am,"

With regard to the right methods and plans, we have nothing new to offer. The following instructions have been issued to all practicing
veterinarians, and our regularly employed Sheep Commission inspectors. (Artell Sutter is one of the latter). A copy is also forwarded to all owners of sheep placed under quarantine on account of foot rot.

"Recommended Procedures For The Eradication of Foot Rot:
When foot rot has been found in a herd of sheep, the entire herd shall be quarantined. All sheep should be set up, and the feet trimmed carefully by paring the horn and sole of the foot so as to expose the diseased tissues. After a careful job of paring has been done, the feet should be immersed in a 30 percent Copper Sulfate solution (two lbs. Copper Sulfate to one gal. water), or a 10 percent Formalin solution (five gal. Formalin to 100 gal. water). This can be done by treating the sheep individually or by driving them through a trough with the solution deep enough for the entire foot to be immersed. All sheep should be put through the foot bath, and the infected ones held in the solution for several minutes. Sheep which show no evidence of foot rot on examination will often show evidence of the disease by showing pain or lameness after going through the foot bath.

The affected sheep should be driven through the foot bath every few days until they are entirely cured. Follow up inspection should be made, and you should recommend slaughter of affected sheep which do not respond promptly to treatment. Otherwise, infection will be retained in the foot of the sheep, and it will show up later when weather conditions favor softening of the foot. The foot rot organisms do not live in the soil for a long period of time. It is retained year after year in the foot of the sheep.

The most serious error is the reluctance on the part of the owner to trim away sufficient amount of the diseased tissue. It is remarkable how soon the horn will grow back if the foot has been properly pared and treated.

Foot rot can be eradicated, and although many owners will be reluctant to comply fully with these instructions, they will thank the inspectors for insisting on a thorough job rather than allowing slipshod procedures, which will result in foot rot showing up year after year. Moreover, the vast majority of owners who are fortunate enough to be free from this disease will appreciate it if we do our work in such a way that their sheep will not be exposed to this serious disease.

The quarantine should not be released until the disease has been completely eradicated."

Foot rot can be eradicated when proper procedures are followed. Moreover, in eradicating foot rot, we are afforded another opportunity of improving the economic position of the sheep industry. There is a great deal of room for improvement with the sheep industry by taking a new look at foot rot and other serious diseases of sheep.

A COMPARISON OF SOME ASPECTS OF VIRAL AbORTION AND VIBRIOsis OF SHEEP
Floyd W. Frank, D.V.M., Ph.D.*
Caldwell, Idaho

Etiology

Ovine viral abortion (OVA) is caused by a virus of the Psittacosis-
lymphogranuloma-venereum (PLV) group.\textsuperscript{15} It is the only virus of this
group known to infect the fetal membranes of sheep.\textsuperscript{1} At least two other
diseases of sheep in the United States are known to be caused by or as-
associated with the presence of PLV viruses. These are polyarthritis of
lambs\textsuperscript{13} and pneumonia of lambs.\textsuperscript{12} Hence, where the complement fixation
test is employed in diagnosis, one must be cognizant of the possibility that
positive titers may result from infections with PLV viruses other than the
agent associated with virus abortion. The method of choice for diagnosis
is the demonstration of the etiological agent in smears of fetal pla-
centas.\textsuperscript{15,14} However, the incidence of infection in a flock is usually much
higher than the incidence of clinical disease (abortions, stillbirths, and
weak lambs).\textsuperscript{1} In some herds a considerable rate of infection, as indicated
by positive placental smears, may be accompanied by an inconsequential
rate of abortion. Thus, although simple isolation or demonstration of this
agent in placental tissues is sufficient evidence of virus infection, it does
not constitute proof that the abortions being manifested in the flock are
due to the virus.

Elementary bodies of virus abortion were demonstrated and \textit{Vibrio fetus}
was isolated from 7.4 percent of 537 fetuses submitted to the Cald-
well Veterinary Research Laboratory during 1959, 1960, and 1961.\textsuperscript{8} In the
flocks involved, an epizootic of vibriosis was superimposed upon virus
abortion, which apparently is enzootic in these flocks. Therefore, to es-
ablish that this PLV virus is responsible for abortions in a particular
flock in a particular year, one must demonstrate that elementary bodies
are consistently present in fetal membranes and also rule out the presence
of other infectious and non-infectious causes.

Vibriosis of sheep is caused by the bacterium \textit{Vibrio fetus}. It is im-
portant to recognize that this agent differs from \textit{V. fetus} isolants associ-
ated with infectious infertility of cattle in cultural characteristics, route
of transmission and pathogenicity. A Belgian worker\textsuperscript{5} has suggested that
within the species \textit{V. fetus} there are two varieties, which he has design-
nated \textit{V. fetus intestinalis} and \textit{V. fetus venerealis}. Table I, reproduced
from the FAO bulletin \textit{Vibrio Fetus Infection of Cattle (1960)},\textsuperscript{11} summarizes
the main differences between these two varieties. \textit{V. fetus intestinalis} is

\begin{table}[h]
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\begin{tabular}{|c|c|}
\hline
\textbf{V. fetus} & \textbf{V. fetus intestinalis} \\
\hline
\textbf{Clinical signs} & \\
\hline
\textbf{Pathogenicity} & \\
\hline
\textbf{Transmission} & \\
\hline
\end{tabular}
\caption{Comparison of \textit{Vibrio fetus} and \textit{Vibrio fetus intestinalis}.
\label{tab:comparison}}
\end{table}

*From the University of Idaho Agricultural Experiment Stations, Veterinary
Research Laboratory, Caldwell Branch Station, Caldwell.
the etiological agent of vibriosis of sheep, but produces only sporadic abortions of cattle. \textit{V. fetus venerealis} causes infertility and abortion in cattle.

In our experience all of 29 ovine isolants studied grew in the presence of one percent glycine but varied considerably in $\text{H}_2\text{S}$ production. Thus, we are in agreement with Florent's observation that growth in the presence of one percent glycine is the best single criterion for separation of the two varieties (the vibrio of infectious infertility of cattle is unable to grow in the presence of one percent glycine).
A closely related species, *V. bubulus*, which differs from *V. fetus* culturally (catalase negative, produces large amounts of H₂S and grows in 2.5 percent NaCl) is apparently a rather common nonpathogenic resident of the genitalia of sheep as well as cattle.² Although the recovery of this agent from aborted ovine fetuses has not been reported, the possibility of its occasional recovery from specimens should be considered.

**Transmission**

Both *V. fetus* and the virus of ovine viral abortion are readily transmitted by oral inoculation of susceptible sheep. This appears to be the natural route of transmission of both diseases.

**Incubation Period**

The incubation period of vibriosis in experimental ewes inoculated orally ranges from eight to 60 days. Highly virulent strains (Marsh and Firehammer Serotype I) appear to produce abortions more quickly than less virulent types.

Experimentally, the incubation period of viral abortion is extremely variable although the minimum time from oral inoculation to abortion appears to be in the order of six to seven weeks. Under natural conditions ewes appear to become infected at lambing time, the infection remaining inapparent until the following pregnancy when it enters into a cycle of active multiplication after the development of the fetal placenta. Lambs can be infected at birth, harbor the agent during growth, and be actively infected when they lamb as yearlings.¹⁶

It has been suggested that because of the long incubation period required to produce abortions in ewes, it is unlikely that viral abortions will result from exposure of pregnant ewes to aborting ewes during a single season. However, exposure of susceptible late lambing ewes to infected early lambing ewes could result in abortions during that season provided the differences in breeding dates allowed sufficient time for incubation (six weeks).

**Signs**

Both vibriosis and viral abortion are characterized by abortions late in the gestation period. Infected ewes lambing at or near term may give birth to stillborn, weak, or apparently healthy lambs.

**Age Incidence**

The published reports from Scotland establish that ewes of any age may be susceptible to infection with the agent of viral abortion. This is also true of *V. fetus* infection. In herds in which viral abortion has been present for a number of years, the peak incidence of infection and clinical disease is among three year old ewes. Such ewes apparently acquire the virus during their first lambing season in the flock (as two year olds), harbor the latent virus during the ensuing nonpregnant period and abort during the next gestation (as three year olds).
The age incidence in ovine vibriosis is extremely variable. However, most severe losses in eastern Idaho, where the disease is endemic, are among younger ewes. Since it is known that older ewes can be highly susceptible, the logical assumption appears to be that in an affected flock where older ewes exhibit only a very low incidence of abortion as compared with that in younger ewes, the older ewes have acquired immunity resulting from previous inapparent infection. Severe losses are sometimes seen in aged ewes. Often these are farm flock replacements purchased from range flocks outside Idaho where management does not entail the confinement of ewes for an extended period during late gestation.

Inasmuch as it has been demonstrated that ewes infected orally while nonpregnant develop significant immunity,\textsuperscript{10} this suggested explanation for the apparent immunity in the absence of a history of prior infection seems reasonable.

**Incidence of Abortion**

The average incidence of abortion reported in OVA infected flocks in Idaho during the three year period, 1959 through 1961, was 3.2 percent (1,807 abortions among 55,661 ewes). The range among the various infected flocks was from 0.7 percent to 14 percent. The data gathered during this study indicate that losses occur annually in infected flocks. In a newly-infected flock, the incidence of abortion may be quite high for two or three years (up to 30 percent).

The incidence of abortion in *Vibrio fetus* infected flocks is often very high (up to 70 percent). However, in contrast to OVA, losses are not usually observed in the same flock in consecutive years. Thus, although OVA does not usually produce spectacular abortion losses in any single year, the annual nature of the problem, contrasted to the sporadic pattern of vibriosis outbreaks, tends to equate the economic importance of the two diseases.

**Geographic Distribution in Idaho**

Most sheep operators in Idaho lamb in sheds. Hence, the ewes are in close confinement at lambing time. Such confinement is conducive to spread of viral infection which, as has been previously stated, is commonly transmitted orally through the medium of infected fetal membranes and uterine discharges from infected ewes to uninfected ewes. These ewes then harbor the latent infection through the ensuing nonpregnant period until the following pregnancy when the infection becomes active. Thus, under such management the maintenance of the infection in the flock is virtually assured. Investigations of the geographic distribution of OVA in Idaho have shown that this disease is widely distributed (Figure 1). One might theorize that virus abortion would be prevalent wherever lambing in relatively close association with other sheep is practiced. Conversely one might assume that where range lambing is practiced, this infection would be of little importance.

The distribution of vibriosis outbreaks diagnosed in Idaho during the years 1956 through 1961 is shown in Figure 2. The larger number of
Figure 1. Distribution of known OVA infected flocks in Idaho.

Figure 2. Distribution of vibriosis outbreaks diagnosed in Idaho.
outbreaks diagnosed in eastern Idaho appears to be related to the practice of feeding ewes in relatively close confinement for a long period during late pregnancy. The number of outbreaks is much lower in western Idaho where ewes are on range or pastures until lambing time.

In any given year, a number of outbreaks are diagnosed in some area of the state. The distribution of these vibriosis outbreaks in an area is often such that spread between flocks cannot be explained by contact transmission, water drainage, or human traffic. Recently, workers at the Caldwell (Idaho) Veterinary Research Laboratory have implicated the magpie as a probable vector. Serotype I *V. fetus* recovered from the feces of magpies seven days after oral inoculation produced a high percentage of abortions among ewes inoculated orally with pure cultures or with feces collected from the infected magpies. These workers also recovered Serotype I *V. fetus* from a magpie 39 days after oral inoculation. These findings appear to explain logically the transmission between flocks in a particular area in a single lambing season.

**Reservoir of Infection**

The importance of the vibrios which have been recovered from bile of ewes and lambs remains in doubt. In order for an animal to qualify as a true carrier of vibriosis, it would have to be demonstrated that such an animal was carrying fully virulent *Vibrio fetus* at a time when other ewes were susceptible (the last half of the gestation period) or one would have to demonstrate that low virulence isolants recovered from such animals have the ability to assume full virulence upon introduction into susceptible sheep. To date only a few bile isolants have satisfied these requisites. Most of the isolants recovered from ovine bile in Idaho have been of unusual or uncataloged serotypes and, where transmission trials have been attempted, have been found to be nonpathogenic or of extremely low virulence. Some of the bile isolants recovered at the Montana Veterinary Research Laboratory are of common *V. fetus* serotypes and have produced abortions when used to challenge pregnant ewes. Recoveries of such organisms have been made at every season of the year, including February and March, when carrier ewes could constitute a hazard to susceptible contact ewes. The duration of gallbladder infection in naturally infected ewes is not known because in many instances the ewes came from flocks without history of vibriosis or were obtained from an abattoir, but in one instance recovery was made six months after the time of probable exposure.

The problem may in part be due to lack of adequate criteria for identification of *Vibrio fetus*. As recently as 1953 it was found that some of the isolants which had been assumed to be *V. fetus* were in fact *V. bubulus*, an apparently nonpathogenic vibrio which was first described and named in that year. At the present time there appears to be considerable justification (previously cited) for further separation of the species *V. fetus* into two varieties: One which causes infectious infertility and abortion in cattle, the other the etiological agent of vibrionic abortion of sheep which causes only sporadic abortion in cattle. Thus, it does not seem unlikely
that we may find that some of the bile isolants are actually distinct varieties, or species quite different from the agent of vibrionic abortion of sheep.

However, there is evidence which suggests that the agent of vibrionic abortion of sheep is capable of establishing residence in the ovine gall bladder. This evidence also suggests that changes associated with loss of virulence occur during prolonged residence in the gall bladder. Since most bile isolants have not been found capable of assuming full virulence on introduction into susceptible pregnant ewes, it seems possible that the changes associated with reduced virulence are not readily reversible, and hence that most bile carriers may be of little importance in the epidemiology of the disease. An equally acceptable hypothesis, which is in keeping with the very erratic pattern of repeat outbreaks, would be that even if only a few of the bile residents retain virulence, or the property of being able to revert to full virulence, they could account for the outbreaks which do occur.

Idaho workers have recovered a number of catalase-positive vibrios from bile and feces of magpies not previously experimentally inoculated with the organism. Sheep inoculation trials with such isolants have not produced abortions and further, the magpie isolants are not serotype I (the serotype usually associated with vibriosis epizootics). Thus, although the magpie has been implicated as a vector of the disease, its status as a reservoir of infection is in doubt.

The agent of OVA is apparently able to maintain itself in a flock of sheep and is transmitted from flock to flock through the medium of latently infected sheep.

Prophylaxis

Immunity following a single injection of oil adjuvant OVA vaccine was shown to produce a significant degree of immunity to challenge with live virus which persisted for 30 months. Serum neutralizing antibody was present at 36 months but absent at 48 months. It was concluded that a single vaccination of ewes in the autumn of the second year of life can be expected to prevent abortions for at least three and possibly four lambing seasons.

The continuous feeding of 80 mg. of chlortetracycline per ewe daily from 10 days prior to inoculation with viral infected fetal tissues until lambing was found to be highly effective in preventing abortions among pregnant ewes. The ewes were infected orally during the third month of the gestation period. The practicability of this method of prophylaxis under field conditions has not been tested.

An adjuvant type vaccine has been shown to be effective in immunizing ewes against vibriosis. It is not known if the duration of protection from such a vaccine extends beyond one year. Immunity appears to be conferred against the homologous serotype only. The feasibility of a bivalent vaccine against serotypes I and V (the most commonly encountered serotypes) is being investigated. Most outbreaks in Idaho have been due to infection with serotype I V. fetus. Serotype V has not been encountered in outbreaks of abortion in Idaho. V. fetus isolants which did not fall into any of the
established serotypes have been recovered from several Idaho outbreaks. However, losses in these flocks have been much less severe than are usually encountered in flocks infected with serotype I isolants.

The currently available vaccine (serotype I) would presumably not protect against these unusual \textit{V. fetus} serotypes. However, because of the lower incidence of abortions produced and the relative infrequency of occurrence of such outbreaks, it is felt that this does not constitute a serious objection to the routine use of the serotype I vaccine.

The available evidence indicates that antigenic variation does occur and that outbreaks due to "variant" strains which are antigenically different from the vaccine strains will continue to be encountered. Our evidence indicates that in Idaho, the serotype I organism predominates. Although knowledge regarding duration of immunity beyond the first pregnancy following vaccination is not available, it is known that the highest incidence of vibriotic abortion in Idaho is among yearling ewes. Hence, assuming protection only during the pregnancy immediately following vaccination, annual vaccination of replacement ewes only should result in a significant reduction in annual losses due to vibriosis. This logic probably would apply only to areas where the infection is endemic, since it assumes that most ewes in such an area are exposed some time during their productive life and develop immunity as a result of natural exposure. This assumption is in keeping with epidemiological observations regarding age incidence and immunity of Idaho ewes.

Chlortetracycline, 80 mg. per ewe daily, fed continuously several days before experimental oral inoculation with \textit{V. fetus} until lambing, was shown to be effective in prevention of abortions.\textsuperscript{7} Epidemiological data, which relates infection to confinement of ewes in advanced pregnancy suggests that continuous feeding of this level of chlortetracycline during this period of confinement could be effective in prophylaxis of the disease.

\textbf{Summary}

Ovine vibriosis and ovine viral abortions are similar in that:

1. Both are transmitted orally.
2. The incidence and distribution of both diseases are related to management. OVA is prevalent in flocks where ewes are confined at lambing time. Ovine vibriosis is seen primarily in flocks in which ewes are confined for an extended period during the last half of gestation. The essential difference is that confinement must occur sufficiently in advance of lambing to allow infection with \textit{V. fetus} and subsequent abortion during the single pregnancy. Since infection with OVA is followed by abortion in the succeeding gestation period, the only management practice necessary for perpetuating the disease is simple contact at lambing time.
3. Clinical signs are the same (abortion, stillbirth, and premature lambs).
4. Vaccine or antibiotic prophylaxis can be used in both diseases.
5. In both diseases, sheep appear to be the reservoir of infection. This is less well established for vibriosis than for OVA.
The two diseases differ in:

1. Etiology.
2. Incubation period. The incubation period of \( V. \textit{fetus} \) is relatively short while that of OVA is quite long.
3. Age incidence. While ewes of any age may be susceptible to either infection, the highest incidence of viral abortions is usually among second lambing ewes. Although the age of incidence of ovine vibriosis is extremely variable, the highest incidence is usually among first lambing ewes.
4. Flock incidence of abortion. The abortion rate in flocks affected with ovine vibriosis is usually much higher than that due to OVA. However, losses due to OVA are experienced annually in an infected herd while repeat outbreaks of ovine vibriosis are relatively uncommon.
5. Vectors. The sheep appear to be the vector of OVA while recent evidence suggests that, in addition to sheep, carnivorous birds can be vectors of ovine vibriosis.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SHEEP AND GOATS


Your Committee this year received a number of requests for publication of situation reports and presentation of papers covering not less than ten transmissible diseases. The requests and topics were carefully screened. To lay stress upon those diseases appearing to be of greatest importance at the moment, three situation reports were accepted and edited for publication in the 67th Annual Proceedings. A fourth situation report will also appear and has already been presented from the floor by Dr. R. L. Hourrigan. The latter situation report was presented to the general session in this manner due to its current interest. As all situation reports and papers, of which two were presented by Drs. Hardy and Frank, will all become portions of the proceedings they are excluded from the committee report at this time. The Committee believes that certain transmissible diseases are of sufficient importance to require resolutions (referred to the Committee on Resolutions). See pages 46 to 49.

REPORT OF PROGRESS AND RESEARCH ON SCRAPIE

Scrapie Outbreaks During Fiscal Year 1963

During fiscal year 1963, there were eleven scrapie-infected flocks reported in six States. The infected flocks, source flocks, exposed sheep, and their immediate progeny slaughtered as a result of these outbreaks involved the payment of Federal indemnity on 131 claims for 4,754 sheep slaughtered from nine States. Federal indemnity of $111,650 and State indemnity of $35,965 was paid.

At the end of the year, there were some 638 flocks of approximately 231,829 sheep in 26 States under surveillance for scrapie. This is a decrease from the previous year when 1,236 flocks were under surveillance.

Illinois. There were four scrapie outbreaks reported during fiscal year 1963 bringing the State total to 28—more than any other State. Infected flock Illinois No. 25 was reported by the owner. Suffolk ewe No. 491 had been purchased from another flock (Flock No. 27) and was showing signs of scrapie 36 months later. The flock of 49 sheep in LaSalle County and approximately 532 exposed sheep or their immediate progeny
in some 20 Illinois flocks were slaughtered. No interstate movements were involved.

The outbreak in flock No. 26 containing 58 sheep in Champagne County was found on routine inspection. The infected Suffolk ewe was not registered. She had been purchased 18 months earlier along with the entire "source flock." No additional sheep were considered exposed.

The infected Suffolk ewe in flock No. 27 was reported by the owner. The large De Kalb County flock of some 1,000 sheep had included those from a number of possible sources of the disease; however, infected ewe No. 122N had been imported from Canada 31 months prior to showing signs of scrapie. The flock included sheep of the Suffolk, Hampshire, Chevoit, and Cotswold breeds. The outbreak occurred just prior to the end of the fiscal year and slaughter was pending on June 30.

Infected flock No. 28 was reported by a veterinary practitioner. Suffolk ewe No. 230 had been bred in a Tazewell County flock. The flock of 179 sheep was slaughtered as were exposed sheep and their immediate progeny.

**Virginia.** Three infected flocks were reported during the fiscal year. All infected sheep were Suffolks. Infected flock No. 5, in Augusta County, was reported by a veterinary practitioner. Ram No. A590 was showing signs of scrapie 24 months after entering the flock of 152 sheep. There were no additional exposed sheep to be slaughtered.

Infected flock No. 6, in Rockingham County, was reported by the owner who suspected sheep scabies. Ram No. 79E had been bred in Virginia infected flock No. 2 found to have scrapie in July 1961. The Rockingham County flock contained 75 sheep. There were no additional exposed sheep identified as subject to slaughter.

Infected flock No. 7, in Albemarle County, was reported by a county agent as possibly having scabies. The infected ram, No. 27H, had also been bred in flock No. 2 and was showing signs of scrapie 30 months after entering the Albemarle County flock of 167 sheep. There were no additional exposed sheep to be slaughtered.

**Texas.** Infected Suffolk ewe No. 27 in Texas infected flock No. 3 had been bred by the Hamilton County owner. The disease was reported by the owner who felt that he had seen other sheep with the disease in his flock of 39 sheep. Ewe No. 301, previously sold from the flock, was found to have scrapie in infected flock No. 4 (to be reported in fiscal year 1964.) Recommended eradication procedures are being followed.

**Iowa.** Iowa's second infected flock was reported by a veterinary practitioner. Suffolk ram No. 48-59 was showing signs of scrapie 21 months after entering the Carroll County flock of 75 sheep. The infected flock and approximately 70 exposed sheep in eight Iowa flocks were slaughtered.

**Missouri.** Infected flock No. 3 was reported by a veterinary practitioner. Suffolk ewe No. 57 was bred in the Clay County flock of 125 sheep. Exposed sheep and their immediate progeny slaughtered included approximately 413 sheep in 32 Missouri flocks, 30 in nine Kansas flocks, and some 173 in eight Texas flocks.
West Virginia. The second infected flock in the State was reported by a veterinary practitioner. Suffolk ram No. 13 had been purchased from another West Virginia flock and was showing signs of the disease 27 months after entering the Marion County flock of 22 sheep. Recommended eradication procedures were followed.
DIARRHEAL DISEASES OF NEWBORN PIGS
D. K. Sorensen, D.V.M., Ph.D. and H. W. Moon, D.V.M.*
St. Paul, Minnesota

Diarrheal diseases are regarded as one of the leading causes of death in newborn pigs. The magnitude of this problem is not fully known since accurate morbidity and mortality data are not available. In recent years newer therapeutic agents and other methods of control have reduced the losses, but this group of diseases is still considered one of the major disease problems which confronts the swine industry. Our knowledge of the causative agents of diarrheal diseases still has many deficiencies and there are differences of opinion; nevertheless, an attempt will be made to discuss this complex of diseases according to the etiology. The scope of this discussion will be limited to enteric disease from birth to eight weeks.

The nature of this paper will be a review of pertinent literature, a report of some experimental studies and clinical observations on diarrheal diseases of newborn pigs.

BACTERIAL INFECTIONS

Escherichia coli (E. coli) Infections

The role of E. coli in diarrheal disease of newborn pigs has not been unequivocally established. Evidence to support its association with diarrheal disease of the newborn pigs is increasing each year. Saunders, et al. in 1960,1 reported the results of investigations on 58 enzootics of disease in piglets, in which E. coli was considered to be the major cause. These authors were able to associate certain serotypes of E. coli, with a clinical disease syndrome. In a later paper, Saunders et al. in 1963,2 reported reproducing the disease in newborn pigs with two serotypes of E. coli [08: L87(B?)K88(L) and 0141 ab:K85(B)K88(L)]. All pigs orally infected with one or the other of these isolates developed a diarrhea and approximately 50 percent died.

The clinical syndrome diagnosed in Minnesota by the authors as "coli-form infection" affects pigs mainly during the first week of life. The disease usually has a sporadic pattern within a herd of swine. In certain enzootics nearly all pigs on a premise sicken in a matter of hours after birth. In others only certain litters or portions thereof are affected. In the majority of cases seen, the disease occurs first as a mild enteric disturbance in the newborn pigs. Litters farrowed in the same house during the next several days are usually more severely affected. The morbidity within a herd is extremely variable, ranging from less than 10 percent to...
nearly 100 percent of the newborn pigs. In certain instances there is spontaneous remission of the disease on a premise while in others the disease is seen again during subsequent farrowings.

In the cases observed by the authors dams and feeder pigs have not developed clinical signs. There have been instances of concomitant mastitis in dams nursing affected pigs. In contrast to transmissible gastroenteritis (TGE) the spread of "coliform infection" to other newborn pigs in a central farrowing house is generally rather slow or does not occur.

The signs observed are usually a yellow to gray or white semifluid diarrhea. There is subsequent dehydration. Vomiting is seen on occasion. Severely affected individuals usually die in 24 to 48 hours. Certain individuals recover spontaneously, others respond to treatment with broad spectrum antibiotics, nitrofurans and intestinal astringents. Recovered pigs may appear normal or in certain instances are unthrifty and reach market weight after prolonged feeding periods.

Necropsy findings are generally dehydration, and soiling about the perineal region with semifluid fecal material. There is usually a.catarrhal gastroenteritis. The intestines may be distended with fluid and gas. There is frequently congestion of the mesenteric vessels. Gastritis is a variable finding. There has been focal ulceration (0.5 - 1.0 cm. in diameter) of the fundic mucosa in several cases. In many cases there are no apparent gross lesions other than dehydration. In general, the clinical syndrome seen in Minnesota is similar to that described by Saunders et al., in Great Britain.

A study is presently underway to determine if certain strains of \textit{E. coli} are consistently associated with "coliform infections" of the newborn pig in Minnesota. This study involves serological 0 grouping of \textit{E. coli} isolates from clinical material submitted to the Diagnostic Laboratory at the College of Veterinary Medicine, University of Minnesota. During the past two years 160 isolates from 86 individuals representing 40 separate enzootics affecting pigs less than eight weeks old have been studied. Approximately two-thirds of the animals studied were dead when presented to the laboratory. Isolates from animals noticeably decomposed at necropsy have not been included.

The results of this 0 grouping are as follows:

<table>
<thead>
<tr>
<th>\textit{E. coli}:</th>
<th>Number of Isolates</th>
<th>Number of Enzootics Where Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 undetermined</td>
<td>58</td>
<td>23</td>
</tr>
<tr>
<td>08</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>0101</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>020</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>belonging to 36 other 0 antigen groups</td>
<td>47</td>
<td>29</td>
</tr>
</tbody>
</table>

Those isolates designated as 0 undetermined are not agglutinated in any of the 146 0 antisera presently available. The 0 antigenic relationships among isolates presently 0 undetermined is under investigation.
There are frequently several groups of \textit{E. coli} associated with a single enzootic of "coliform infection."

\textit{E. coli} isolated from newborn pigs with TGE, \textit{Cl. perfringens} type C enterotoxemia, hypoglycemia, and injectable iron toxicity, have been grouped. Over one-half of these isolates are 08 and 0 undetermined.

This syndrome "coliform infection" is the most prevalent form of diarrhea seen in young pigs in Minnesota. There are obviously many other factors associated with its causation. The role of stress, and management is important but it appears likely to the authors that \textit{E. coli} is a primary agent.

\textbf{Enterotoxemia}

\textit{Clostridium perfringens} type C enterotoxemia of the newborn pig has been reported in Great Britain,\textsuperscript{3} and Hungary.\textsuperscript{4} The British workers reproduced the disease in newborn pigs by feeding culture and/or toxin.\textsuperscript{5} The Hungarian workers report successful vaccination trials using the Type C toxoid administered to pregnant sows.\textsuperscript{6} This same group has also reported on the efficacy of type C antitoxin administered to pigs during the first day of life. There has been mention of this disease occurring in the United States.\textsuperscript{7}

\textit{Clostridium perfringens} type C enterotoxemia of the newborn pig follows an acute course, frequently terminating fatally within twenty-four hours after the onset of clinical signs. The first sign observed is usually a diarrhea, which frequently becomes blood tinged in a matter of hours. Erythema of the perineal region and tail, and dehydration are prominent subsequent findings. The gross lesions are necrosis and hemorrhage in the intestinal mucosa. These changes, on occasion extend to the serosal surfaces, and are confined chiefly to the jejunum. There is frequently an abrupt line of demarcation between normal and affected tissue. The disease has occurred in Minnesota in at least five separate enzootics, affecting pigs from one day to four weeks of age. The first of these enzootics will be described in an article to be published soon.

Briefly, the diagnosis is established by demonstrating the typical lesions at necropsy and the isolation of type C, \textit{Cl. perfringens}. In certain cases it is possible to demonstrate preformed toxin in the intestinal contents or in ascitic fluid. The toxin is identified by serum neutralization tests in mice.

\textbf{Salmonellosis in Newborn Pigs}

Salmonellosis in nursing pigs is infrequently encountered. When it occurs it usually develops in older nursing pigs from six to eight weeks of age.

In general, salmonellosis in swine is most common in the three to six month age groups. The reason for the infrequent occurrence of clinical salmonellosis in nursing pigs has not been studied. Since we obviously have many asymptomatic carriers in our breeding animals there must be considerable opportunity for exposure to nursing pigs. In the outbreaks of salmonellosis in older nursing pigs there frequently is a history of poor
management and concomitant disease such as hog cholera or other forms of stress.

In a survey of the cases from the past four years at the Diagnostic Laboratory at the College of Veterinary Medicine, University of Minnesota, salmonella was isolated from three cases of disease in nursing pigs. In one instance it was incriminated as the primary agent in diarrheal disease. The other two cases were sporadic deaths and did not involve an enzootic of diarrheal disease. In these two cases generalized infection was present.

Shigellosis

Shigellosis in newborn pigs has been reported from Missouri. In the case reported, 75 percent of the pigs from 100 sows died. Affected pigs developed a diarrhea and began vomiting at about two weeks of age. The causative agent was identified as *Shigella dysenteriae*.

This disease is not known to occur in Minnesota. Shigellosis certainly is not a major cause of diarrheal disease of the newborn pig.

**MYCOTIC INFECTIONS**

There have been reports of infections of the alimentary tract of swine with species of *Candida* and *Rhizopus*. These organisms appear to have a predilection for the oral, esophageal and gastric mucosa. In two reports, the infections were associated with diarrheal disease of newborn pigs. The role played by these organisms, whether primary or secondary infections of the swine alimentary tract, is not known.

Mycotic infections are not recognized as a major cause of diarrheal disease of newborn pigs in Minnesota. The possibility of such infections (particularly with species of *Candida*) after prolonged oral administration of antibiotics must be considered. Mycotic infection of the intestinal tract of turkeys and man under such conditions are not uncommon.

**VIRAL INFECTIONS**

**Hog Cholera**

Hog cholera virus is another cause of diarrhea in young pigs. Infection with virulent strains of virus results in a clinical picture that is usually not confused with the other diarrheal diseases of young pigs.

In contrast, the syndrome resulting from infection with a low virulent virus varies; diarrhea and unthriftiness may be the principal clinical signs. Hog cholera may occur in suckling pigs after the sows are vaccinated with a modified live vaccine. Diarrhea, due to low virulent hog cholera virus has occurred on farms where large numbers of pigs were raised and there were pigs of all ages, some of which were vaccinated and others were not. It has also been observed on farms where the history indicated there was no hog cholera immunization.

This is illustrated by a clinical case in which, one to three weeks after vaccination of the sows, nursing pigs developed a diarrhea, quit eating,
became unthrifty and died. At the time of the investigation, 18 pigs had died, the remaining 20 were unthrifty and had a diarrhea. Temperatures ranged from 101 to 105. Necropsy findings were principally those of a few petechial hemorrhages on the kidneys and an enteritis.

This enzootic is typical of several that have been encountered. Animal inoculation tests have confirmed the diagnosis of hog cholera.

**Enteroviruses**

There have been reports of isolation of swine enteroviruses in recent years from Europe,\textsuperscript{13,14} Great Britain,\textsuperscript{15,16,17} Canada,\textsuperscript{18} and the United States.\textsuperscript{19,20,21,22} In general, these agents are small in size (25–40 μm), ether resistant, able to grow and produce a cytopathic effect in swine kidney tissue cultures, and capable of multiplication in the alimentary tract of swine.

Many of these enteroviruses may at present be called "orphans" as they are not known to be associated with disease. Certain "orphans" have been used in unsuccessful attempts to produce disease in swine, while others were isolated from healthy swine and there is, as yet, no evidence to indicate they are pathogenic.

Those porcine enteroviruses shown to be pathogenic have the ability to produce an encephalomyelitis in either suckling or colostrum deprived pigs. Viruses in this group have been isolated from normal pigs, pigs with diarrhea, and suckling pigs with central nervous disturbances.

Izawa \textit{et al.},\textsuperscript{23} report experimental reproduction of a diarrhea in young pigs with an enterovirus isolated from a clinical case of diarrheal disease. This is the only such report the authors are aware of. The agents which they describe produced both gastroenteritis and encephalomyelitis in newborn colostrum deprived pigs. These agents did not produce disease in suckling pigs.

The authors have on occasion seen encephalomyelitis in suckling pigs at Minnesota. These have occurred both during and after enzootics of diarrhea in the affected litters. In such cases sows and older swine have remained unaffected.

In summary, there is very little direct evidence at present to indicate that enteroviruses are a major cause of diarrheal disease in the newborn pig. There is, however, abundant evidence that enteroviruses are widely dispersed in the swine population. For this reason, the relationship of enteroviruses to diarrheal diseases of the newborn pig would seem to offer a fruitful field for future research.

**Transmissible Gastroenteritis (TGE)**

TGE virus is an important cause of diarrheal disease in the newborn pig but will not be discussed due to limitations of time.

This has been a rather superficial treatment of enteric disease of the nursing pig. It also represents an over simplification of the etiological factors involved. Obviously, other factors are important along with the agents mentioned. It is recognized that there are undoubtedly other agents yet to be discovered.
REFERENCES


ROLE OF THE NATIONAL SPF AGENCY IN THE
CONTROL OF SWINE DISEASES

Willard Dielschneider

Conrad, Iowa

Mr. Chairman, Members of The United States Livestock Sanitary Association, Gentlemen: I am flattered that you have asked me, a farmer, to speak to your Association. I hope that I can give you the information that you wish. I could probably do better if you were to come to me on my farm with questions. I find that I can give out information much better in that way than I can up here trying to speak to you.

I am going to begin today by giving you a little bit of history behind the SPF movement. I will tell you a little about how it all came about and then I will go on and point out to you the way that the National SPF Accrediting Association fits into the picture. As you probably all know, Dr. George Young, working with the University of Minnesota, and the Hormel Institute of Minnesota, devised the Hysterectomy process of obtaining disease-free pigs for experimental work. Doctor Young later moved to Nebraska and the University of Nebraska at Lincoln, where several years ago he started a disease-free herd for the University. Some of these hogs found their way back to the farm where the University used them to gather data on their performance. In 1959 the first commercial Laboratory was started for the purpose of doing custom hysterectomies for farmers with the idea that in this way farms infested with Virus Pig Pneumonia and Atrophic Rhinitis could depopulate their farms and buy back clean stock to repopulate their farms. When the SPF program moved back to the farm it presented a problem to the industry in that it needed policing to see to it that the scalper or fly-by-night operator did not ruin the program before it got off the ground. Before this time the SPF hog had remained in control of a University. Now, however, any farmer could purchase or produce SPF hogs. Many of the farmers did not know how to handle this hog and it was felt that an education program as well as a policing force was needed.

Because of this, two groups soon came into existence. One of them was a veterinary group known as The National Swine Repopulation Association (NSRA). This group was headed at that time by Doctor Bert Combs who was also the founder of the first commercial laboratory, C & G, at Conrad. Under the direction of Doctor Combs and Doctor Maynard Spear of Iowa State University, the first commercial producers of SPF swine were also formed into a group known as The Central Iowa Swine Repopulation Association (CISRA). This group was formed in order to educate the producer and also for the purpose of checking on the herds to see that they maintained certain health standards as to sanitation and isolation that were deemed necessary to keep SPF swine. Rules for these practices were formed by both organizations which were very similar. Both organizations
worked toward certification or accreditation programs for SPF swine. Because of the fact that the central Iowa group was closer to the problem, it seemed to progress toward this end a little faster than the National group. Before the central Iowa group started an actual accrediting program the SPF movement had spread throughout the state and it was decided by a group headed by Doctor Williamson of Shenandoah, that the accrediting should be done on the state level. For this reason then, the Iowa Swine Repopulation Association (ISRA) was formed, and this is the group that started issuing accreditation certificates to SPF swine that could meet the health standards as set up by the Central Iowa Group and the National group. The leadership and organizational work that had gone on in the central Iowa group was used as a basis for the state group.

There was one difference between the CISRA, ISRA, and the National Association that at that time caused some friction. The National Group insisted on tying performance standards into the SPF program and by so doing would eliminate herds if they did not meet these standards. The other two groups, being producers with quite a lot of money involved in a new program, did not wish to face the possibility of being ruled out because their hogs did not meet certain weight standards, and so they argued that the SPF program should be concerned only with the health of the animals. Since there was at that time no direct tie between the two groups, it was hard to communicate back and forth between the two. For this reason the Iowa group suggested that there should be producers represented in the National organization if it was to be the rule making body. By this time the movement had spread to several states so the Iowa group called in producers from all known SPF states with the idea of approaching the National with a proposal that there be included in the National a body which would be known as the Producers Council to NSRA and that this body would be composed of producer representatives of each SPF state. This group then would meet with the NSRA board and the two bodies working together would develop the rules of the National. This proposal was presented to the NSRA and accepted by them. Responsibilities were designated to each group. It was decided that the veterinary group or NSRA should be responsible for setting up the health standards of the group. It was agreed that the performance standards previously set by NSRA would be dropped from compulsory to voluntary basis. The National Producers Council was given the responsibility of being the policeman of the organization and to see to it that the health rules set up by NSRA would be lived up to by the producers. For this reason then, the National Producers Council formed what is now known as the National SPF Accrediting Agency. This group would do the accrediting of SPF swine in such a way that the rules established could be kept in force.

I will tell you some of the things that the National Accrediting Agency uses as checks to determine whether a man's herd is truly SPF. First of all we must know the source of the SPF stock with which he got his start. If this is laboratory stock, then we must have a statement from that lab to the effect that this man did get stock from that source. This we call a CR or Certificate of Recommendation from the lab. If he purchased stock
from another accredited herd to get his start, then we must have a copy of
the certificate which serves as a transfer of stock from the herd of origin
to his herd. If we have all of these on his herd then he is considered to
have had a start with clean hogs. The next thing then that enters the pic-
ture on this man's herd is a health and disease form from his veterinari-
an. This gives us information as to the clean up and period of depopula-
tion, management practices, isolation practices, etc. One of these reports
from the man's veterinarian is due to the National office every 90 days. If
these reports are not in the office or current there will be no certification
done on that herd. Another thing that is required along with the health and
disease report is a brucellosis validation on that herd.

The next thing required of a producer is that he take 10 percent (not
less than seven nor more than ten) of the hogs out of each crop, through a
slaughter inspection at a pre-arranged slaughter point on a pre-arranged
date. These hogs out of his herd are then examined for presence of the two
diseases, VPP and AR. If there are any visual suspects at this time,
samples of the snouts and/or lungs must be sent to the pathology depart-
ment for that area (usually State Labs.). These cultures are then examined
and tested. Should the laboratory report be negative, then the herd qualifi-
cies for certification. If they should be positive, then the herd is disquali-
fied. If there were suspect samples sent to the laboratory, that herd is
placed under suspension until the laboratory report is received.

When a herd meets all of the preceding requirements, then this herd
is issued a card which states that this is an accredited herd. When this
card is issued then a man may sell accredited SPF stock. Any stock which
he sells can then receive a certificate. All of the above must be up-to-
date and current, including the slaughter card.

Should a herd at any time become disqualified for any of the reasons
that I gave before, the only way that that herd can be re-accredited is to
get a clean start either through the laboratory or through the purchase of
stock from another producer.

As I stated earlier in my talk, there are no compulsory performance
standards in the SPF program. Each producer, however, is urged to
gather all of this kind of information that he can on his stock in whatever
way he can. We recommend that the purebred producer should take ad-
vantage of PR programs, boar test stations, on the farm testing programs
sponsored by some of the breeds, or his own on the farm testing program.
It is our feeling that the man who takes the time and effort to gather this
kind of information will be able to demand the higher price for the stock
he is offering.

There are other things that can disqualify or suspend an SPF herd.
The presence of Mange or Lice, for example, are taken to mean that that
herd has had an exposure to other swine and will suspend that herd until
this condition is cleared up and the herd has gone through a slaughter in-
spection to prove that there was no VPP or AR contracted through the ex-
posure. Bloody scours found in an SPF herd will disqualify that herd even
though VPP or AR are not present. Other types of scours or dysentery
will suspend that herd until the condition is cleared up.
At the present time there are nine states that are accrediting through this office. There are out of these states a number of members in the Association. To date out of these herds there have been a few herds that have gone down because of re-infestation with either one or both of the diseases.

At the present time there is a move on to simplify the bookwork that is involved with the program. There is under consideration a possible working agreement between our organization and the existing purebred associations whereby some of the bookwork involved might be carried on in connection with purebred registry. There is also under consideration a program for the commercial man who is not interested in selling breeding stock but is rather interested in selling feeder pigs, for example. Under this kind of a program it is thought that there would not be individual certificates issued for each pig but rather a group certificate for each group of pigs that are sold. These pigs then could not be used to repopulate another farm but would merely be a source of clean feeder pigs for the man who is in that kind of a program.

Careful study is being made of each of these proposals to be sure that the simplification does not go so far that the disease control of these herds is lost. We feel that we have a good program going and we do not wish to ruin it now. Of course the program could not have gone as far as it has if it had not been for the cooperation of the various Universities and Colleges throughout the country. Two of these that have been especially valuable have been the University of Nebraska, and the University of Iowa. Much time and effort has gone into this program by some of the personnel at these two universities. The extension personnel have been used as consultants and advisors in setting up this program. We hope that this cooperation can continue. We feel that with the use of the SPF program it will be possible to make the American hog the healthiest hog in the world.
AN UNUSUAL OUTBREAK OF AUJESZKY'S DISEASE IN SWINE
J. Robert Saunders, Donald P. Gustafson, Harvey J. Olander and Russell K. Jones*

INTRODUCTION

Previous reports\(^1\,2\,3\) have indicated that Aujeszky's disease (Pseudorabies) (AD) occurs frequently in midwestern swine but is symptomless except in suckling pigs. Shope\(^4\) states, "Pseudorabies as it occurs naturally in older swine in the United States is a 'silent' subclinical infection." In Europe and Asia, however, Aujeszky's disease virus (ADV) has caused high morbidity and mortality in older swine.\(^5\,6\,7\) In Europe pseudorabies has been recognized as a cause of early embryo mortality. Stillbirths and abortions with accompanying illness occurred among sows.\(^8\,9\) That infection with this virus apparently has not produced similar disease in North American swine may be due to differences in husbandry and nutrition or to strain differences of the virus or it may be that such infections have been confused with other diseases and therefore not recognized.

This is a report of three epizootics of AD encephalitis in swine of all ages with ancillary virus isolation studies and histopathological observations.

CASE REPORTS

Encephalitis in swine in three herds on five farms was investigated from November 1962 through March 1963. These farms were within a five mile radius in Carroll County, Indiana in an intense swine producing area. The herds were 'closed' herds with no additions having been made within the past year. All three of the swine herds reported here were family owned and operated as is typical of Indiana production units. The swine were crossbreedings of Hampshire, Yorkshire, and Poland China breeds.

MATERIALS AND METHODS

Except for four specific pathogen free (SPF) pigs used experimentally, the swine examined at the Animal Disease Diagnostic Laboratory (ADDL), Purdue University, were accessions from the three affected herds. These

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Paper No. 2233 journal series, Purdue University, Agricultural Experiment Station, Lafayette, Indiana.

The authors wish to thank the following practicing veterinarians for their cooperation in this study: E. S. Emerson, Delphi, Indiana; S. H. Shipsides, Flora, Indiana; and D. R. Bright, Rossville, Indiana.
pigs were sent to the ADDL on the advice of veterinarians or were obtained on visits to the farms.

Live pigs were examined clinically, bled for hemograms and euthanized. Necropsies were performed with particular attention being given to the central nervous system (CNS). Bacteriologic examination consisted of streaking pieces of various organs on blood, tryptose and brilliant green agar plates. Selenite broth was also used to cultivate bacteria from selected specimens. Qualitative tests for lead and arsenic were performed on some specimens. Tissues for histopathology were fixed in 10 percent buffered formalin, embedded in paraffin, cut at six microns and stained with hematoxylin and eosin.

For virus isolation and identification, tissues were collected as aseptically as possible and primary cell cultures were prepared from some portions. Other portions of tissue specimens, either fresh or frozen at -15 or -35 C were used for the inoculation of rabbits and subculturable cell lines. Occasionally 50 percent glycerol was used as a preservative.

For inoculation of rabbits the tissues, usually CNS, were ground in a mortar with sufficient sterile buffered saline to make a 10 to 50 percent suspension. Penicillin and streptomycin were added to give final concentrations of 1000 units and micrograms respectively/ml. One to two ml. of the suspension was then inoculated into a rabbit subcutaneously. The animals were observed daily for a maximum of one week.

Primary cultures of porcine brain, spinal cord or kidney were prepared by mincing the tissues and suspending in 20 percent normal swine serum in Eagle's basal medium containing 350 mgms percent glucose and one ml. each of glutamine and antibiotics; streptomycin, penicillin, and achromycin-(SPA)/100 ml. These suspensions were put in T flasks or Leighton tubes containing cover slips and maintained for a maximum of three months. For demonstrating inclusion bodies the cover slips were fixed in methyl alcohol and stained with May-Grunwald-Giemsa. Subculturable cell lines used were porcine (PK), monkey (MK), rabbit or bovine kidney, swine embryo lymph node, ovine leucocyte and ovine brain and choroid plexus. The medium used was five to 20 percent calf serum in Eagle's basal medium containing glucose, glutamine and SPA. Inocula consisted of 0.1 ml. of a 10 percent suspension of the various tissues. The cultures were maintained for a minimum of 72 hours to a maximum of two weeks. Harvested tissue culture fluids containing virus were diluted 1:2 in glycerol and stored in ampoules at -35 C. Stock virus was titered out in PK or MK cultures and stored in the same manner.

Serum neutralization tests were carried out in PK or MK cultures. Equal volumes of two fold dilutions of serum, inactivated at 56 C for 30 minutes, and virus dilution containing 50-100 TCID_{50}/ml. were mixed and incubated for one and one-half hours in a 37 C water bath. 0.2 ml. of the mixtures were then inoculated into each of three to five cell cultures and observed daily for seven days for cytopathic effect. Controls consisted of uninoculated cultures, virus titrations and cultures containing serum alone. As a screening test a 1:2 dilution of serum and 10 to 100 TCID_{50} of virus were employed according to the method described by Mackay et al.\textsuperscript{10}
Figure 1. Diagram of Farms A, B and C, Herd "S".

Case 1: Herd 'S': Farms A, B and C

Figure 1 shows the geographical relationships of these three farms. Farm A had a large dairy barn which had been converted to permit the feeding of swine; there was also an isolated woodlot for pasturing bred sows. Farm B had a large shed-type barn divided in half; one half opened into a large enclosed feedlot while the other half opened into a pasture in which sows and gilts were kept. Farm C had a three-story farrowing house to which sows from the other two farms were brought.

In late November 1962 on Farm A, sows and gilts bred for December farrowing were being kept in the woodlot. In the barn were 600 feeder pigs, five months of age. On Friday, November 23rd, when 193 fat hogs were trucked to the local stockyards; slight coughing was observed in the group. The 193 were held until the following Monday for slaughtering at which time three were dead and the remaining 190 were so empty that they were estimated to have lost an average of 10 pounds each.

The remaining 407 were sorted on November 24 with 123 gilts being sold for breeding purposes. These 123 gilts were trucked to the new owner's farm a few miles away on November 25; 32 gilts were then shipped to another farm at Kansas City and the remaining 91 were turned into an open field without shelter. The weather became cooler and by November 27
some gilts were obviously sick with an influenza-like disease. The symptoms noted were coughing, anorexia, vomition, shaking of the head, frequent getting up and down and convulsions. By November 28th seven had died and 20 others were sick. The attending veterinarian made a tentative diagnosis of toxicity, treated with penicillin and sent two dead gilts to the ADDL. At necropsy the ventral body wall was reddened; the gastrointestinal tract was empty and the viscera were generally congested. Tests for lead and heavy metals were negative. Bacterial pathogens were not isolated. The CNS was not examined histologically or frozen for virus isolation. An etiologic diagnosis was not made. Twelve gilts died in this group. Some sick ones were put in with other gilts on the farm. The latter developed a similar sickness in 10 to 14 days, but there were no deaths. Two of the 32 gilts sent to Kansas City were reported to have died with similar symptoms. Some sows which came in contact with the sick ones were reported to have aborted two to three weeks later.

On November 26, because coughing was more noticeable in the feeders remaining at Farm A, the owner began treatment for influenza with sulfathiazole in the drinking water. Some pigs died on November 27 and 28 so the veterinarian visited the farm on November 29 and brought a dead pig to the ADDL. Nine pigs had died by this time. The symptoms noted were coughing, anorexia, vomition, constipation, trembling, tonic spasms and convulsions. Temperatures ranged from normal to 105 F. Approximately three percent of the pigs had nervous symptoms.

The findings at necropsy of the dead pig were edema and congestion of the lungs, pleural and pericardial effusion, epicardial petechiation, empty congested stomach, congestion of the intestines, liver and spleen, peripheral congestion of many lymph nodes and extreme congestion of the meningeal vessels. Bacteriologic examination was negative. Histopathologically there was a diffuse, non-suppurative meningo-encephalitis with swelling and degenerative changes in the vascular endothelium in many organs. The CNS, unfortunately, was not frozen for microbiologic examination.

All the pigs had been vaccinated against hog cholera with at least two different lots of modified virus vaccines. Although gross lesions were not suggestive of hog cholera the microscopic lesions were suggestive so another dead pig was obtained at the end of the episode on December seventh. Deaths now totalled 13. By this time most of the pigs had recovered and were back on feed. The only treatment had been sulfathiazole and good care. The gross lesions found at necropsy were similar to those in the first pig except there was a hemorrhagic enteritis. Some corynebacteria, coliforms and staphylococci were isolated from the viscera. Toxicologic examination was negative. Histopathologically the lesions were similar to those in the first pig. Brain and visceral organs were frozen at -15 C but it was not until four weeks later that a rabbit was inoculated with brain suspension with negative results. Subsequent inoculation of another rabbit with a more concentrated suspension of brain, lymph node, lung and kidney caused death in 78 hours with signs and symptoms suggestive of AD. Tables I and II summarize the deaths in the various lots at the three farms and the methods used to identify the virus.
TABLE I

Aujeszky's Disease in Swine Herd 'S'

<table>
<thead>
<tr>
<th>Farm</th>
<th>Age Group</th>
<th>Deaths/Number in Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>'A'</td>
<td>5 Mo. fat hogs</td>
<td>3/193</td>
</tr>
<tr>
<td></td>
<td>5 Mo. gilts</td>
<td>14/123</td>
</tr>
<tr>
<td></td>
<td>5 Mo. feeders</td>
<td>13/284</td>
</tr>
<tr>
<td>'B'</td>
<td>4-6 Mo. feeders</td>
<td>18/492</td>
</tr>
<tr>
<td></td>
<td>bred sows</td>
<td>1/112</td>
</tr>
<tr>
<td>'C'</td>
<td>nursing sows</td>
<td>2/52</td>
</tr>
<tr>
<td></td>
<td>baby pigs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>birth to 5 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pregnant sows</td>
<td>175/430</td>
</tr>
<tr>
<td></td>
<td>none/64*</td>
<td></td>
</tr>
</tbody>
</table>

*Only 64 of the 112 from Farm 'B' did farrow.

TABLE II

Virus Identification in Swine Herd 'S'

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number</th>
<th>Swine Age</th>
<th>Circumstance</th>
<th>Test - No. positive Cell culture</th>
<th>No. tested</th>
<th>Rabbit inoc.</th>
<th>Serum neut.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4</td>
<td>5 mo.</td>
<td>d. o. a.</td>
<td>---</td>
<td>1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>4 mo.</td>
<td>necropsied</td>
<td>---</td>
<td>0/2</td>
<td>0/2</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6 mo.</td>
<td>isol. c SPF #43</td>
<td>---</td>
<td>0/1*</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6 mo.</td>
<td>isol. c SPF #55</td>
<td>---</td>
<td>0/1*</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>1 wk.</td>
<td>necropsied</td>
<td>9/9</td>
<td>9/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2 mo.</td>
<td>serum samples</td>
<td>---</td>
<td>5/5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Oral and nasal swabs tested.

**Two SPF pigs, one hog cholera susceptible and one hog cholera immune were inoculated with tissue culture fluid but did not become sick or develop serum neutralizing antibody to ADV.

***Source - Purdue University Veterinary Research Farm.

Rats were very plentiful on Farm A, particularly around the buildings but none were trapped for virus isolation. A cat did die suddenly during the outbreak but was not brought to the ADDL for examination. Other livestock were not kept on this farm.

Farm B

Symptoms of illness were first noted on December 15, 1962 in feeder pigs on this farm. This was approximately three weeks after the epizootic began on Farm A. One pig had convulsions and others were off feed and
vomiting. In retrospect, the owner thought some slight signs of illness were apparent about three days before.

There were 604 pigs on this farm. One half of the barn sheltered 112 sows and gilts bred to farrow in February; across the dividing fence were 492 feeder pigs, four to six months of age. On December 17th the veterinarian was called to the farm and noted that the feeder pigs had symptoms similar to those seen previously in pigs at Farm A. Temperatures of the sick pigs ranged up to 105.5. Five pigs had died since the owner first noted signs of sickness two days before. There were 16 sick pigs in a sick pen. Two of the sows had aborted.

One of us (DPG) visited the premises on December 21st with the veterinarian. Some sick pigs were observed to vomit and others were trembling and had convulsions. Two sows were down and unable to get up and one had a convulsion. Four sick pigs were brought to the ADDL. Two were euthanized and necropsied and the other two were put in isolation units for observation.

Of the two necropsied on December 21 gross and microscopic lesions were similar to those seen in the two pigs from Farm A. Brain and spleen were stored at -15 C until inoculated into a rabbit three weeks later with negative results. Small pieces of various tissues had also been stored in ampoules at -35 C. These were inoculated into ovine leukocyte cell cultures in March, 1963 with negative results.

On December 24th two SPF pigs (Nos. 43 and 55—Table II) were put in the isolation units with the two pigs kept for observation. One SPF pig was susceptible to hog cholera and the other was immune. These four pigs were observed daily until March 13th when the SPF pigs were turned out. Temperatures and clinical observations were recorded daily during the observation period. Some oral and nasal swabs were also collected for inoculation of tissue cultures but were negative. Blood samples were collected on occasion for total leukocyte counts and for serology. Both pigs from Farm B had exacerbations of clinical symptoms - temperatures fluctuating from normal to maximum of 105.5 F, anorexia, tremors and nasal discharge - until early in February. After this time, their temperatures were relatively normal and only rarely were there any signs of trembling. The one SPF pig No. 55 had elevated temperatures of 103 - 105 F periodically during January in association with trembling. These signs were not observed later. This pig did develop a significant antibody titer to ADV while the other SPF pig did not.

The two convalescent pigs from Farm B were finally euthanized and necropsied on May 11 but gross lesions were not observed. Occasional glial nodules were present in the cerebrum of the one pig. Foci of acute non-suppurative inflammation were present in the cerebrum of the other pig and resembled that seen in the brains of pigs dying from acute encephalitis.

Another visit was made to Farm B on December 26, 1962 at which time most of the pigs were well. Deaths totalled 18 of the 492. One of the sows previously down was recovering while the second one was not and eventually died. A third sow was down at this time but gradually recovered
and was shipped for slaughter. One week after this visit it was reported that many of the sows were coughing but this subsided after treatment with sulfathiazole. The owner estimated that about 50 percent of the 112 sows aborted. At farrowing time in February, only 64 (57 percent) farrowed while the rest were barren. Serological tests were negative for brucellosis and leptospirosis on the few sows tested in early January. It was therefore assumed that these diseases had not caused the abortions.

**Farm C**

Fifty-two pregnant sows and gilts of a total of 57 were transferred from the woodlot, Farm A, to the central farrowing house during the last week of November, 1962. There was no known contact with the infected feeder pigs on Farm A.

The gilts were put on the top floor of the farrowing house (Figure 1). Farrowing began on the fourth of December and ended on the 17th. On December 24th the owner was first aware of sick baby pigs on the third floor, east side only. A few pigs had died the week before apparently due to natural causes. However, during this week two sister sows had developed agalactia and all their pigs had died; this had not happened before but was not thought significant so these two sows were taken out of the farrowing house. On December 26th a dead pig and a live one, three weeks of age, were brought to the ADDL. Symptoms of a disorder of the CNS were noted and the rectal temperature was 105.5. At necropsy gross changes included congestion of the viscera, peripheral hemorrhage of some body lymph nodes, slight petechiation of the renal medulla and cortex, an empty gastrointestinal tract and marked congestion of the CNS. Bacteriologic and toxicologic examinations were negative. A diffuse non-suppurative panencephalomyelitis with ganglioneuritis and a necrotizing lymphadenitis were the significant histologic findings.

One of us (DPG) visited the premises on December 28. The seven sows on the top floor, west side, were not affected with the exception of one sow that was weak, unable to get up and had fat, listless and ataxic pigs. There were only nine pigs living from the litters of five sows on the east side. One of these sows gave birth to nine pigs but only one was still living. Two live sick pigs were brought to the ADDL. They had a CNS disorder, temperatures of 103.2 and 105.3, hematocrits of 46 and 48 percent, total leukocyte counts of 11,700 and 18,750, and differential leukocyte counts of 30 and 78 neutrophils, 60 and 21 lymphocytes, four and one eosinophils and six and zero monocytes respectively. Partial collapse of the antero-ventral lobes of the lungs, a slight excess of thin, clear pericardial fluid, tenacious gastric mucus, empty intestines, a few renal medullary petechiae and peripheral reddish-brown discoloration of the lymph nodes were observed grossly. Microscopically there was a diffuse non-suppurative meningo-encephalitis with ganglioneuritis. Subsequently, frozen tissues and fluids from tissue cultures inoculated with brain gave positive reactions in rabbits for ADV.

By January 3, 1963, a few pigs on the second floor of the farrowing house had died with similar symptoms and the infection appeared to be
spreading rapidly. On another visit to the farm on January eighth it was found that of three litters noted sick on the previous day, all pigs were now dead. Many pigs had sickened, and many had died during the previous week. Mortality was generally lower in pigs over three weeks of age. Some signs of illness noted in the sows were anorexia, agalactia, weakness, vomition, and occasional convulsions. Seven sick pigs, three to four weeks of age, were brought back to the ADDL for observation and euthanasia. The four pigs still living the next day were examined clinically and all had signs and symptoms of encephalitis. One pig circled to the left; another had opisthotonus and continually backed up. The other two could stand, but when lying down they paddled with their legs, had opisthotonus and preferred to lie on their right sides. The corneal reflex was present in all four pigs. Rectal temperatures were 97.8, 99.6, 103.6 and 104.6. Blood samples were collected, and the pigs were then euthanized. The total leukocyte counts were 11,550, 18,900, 19,750 and 6,650. The gross lesions were very similar to those seen in baby pigs previously obtained from this farm. One pig had small necrotic foci in the liver and spleen and another pig had peripheral hemorrhages in the spleen. A few coliforms and staphylococci were isolated from the viscera of two of the freshly killed pigs. Diffuse non-suppurative meningo-encephalitis was a consistent histological lesion. Pooled brain suspensions inoculated subcutaneously into a rabbit caused death in four days with signs and lesions typical of Aujeszky's disease. This was the first case in which a positive test was obtained. A bacteria-free filtrate of the rabbit's CNS produced death when inoculated into other rabbits. Positive results were also obtained in primary tissue cultures set from the brain and spinal cord but not the kidney of these pigs.

The farm was revisited on February fourth and again in early March. Twenty of the 52 sows were reported to have been noticeably sick during the epizootic. One sow became prostrate and subsequently died. Two others became very weak and were sent to slaughter and one did not pass inspection for use as food. These sows had farrowed an average of eight to nine pigs making a total of 430. At weaning time 255 pigs were moved into the barn at Farm A. Therefore, 175 pigs had died during the course of the disease. It was observed that the mortality rate was much lower in litters three to five weeks of age as opposed to younger ones. The 255 weaned pigs were fairly even in size although there were some runts and also 10 to 15 'camel-backed' pigs. No rhinitis was observed although there was some coughing which may have been due to virus pneumonia. Blood samples collected from five convalescent pigs were positive on the SN test for ADV.

Most of the fat hogs that were first affected on Farm A were marketed by February first and what few stragglers were left were ready for market at that time. There were no condemnations at slaughter.

The farrowing house was cleaned out and well disinfected after the 255 pigs were weaned. The pregnant sows from Farm B were moved in during the first week of February. Farrowing began February 10th and 64 sows farrowed normal litters. Sickness was not a problem in these pigs. Thus
46 to 48 of the 112 bred sows were not pregnant after going through the epizootic of AD.

**Case 2: Herd 'T'**

On January fourth, 1963, three pigs, one month of age, were submitted to the ADDL (Table III). Seventy pigs in this group had died within the past 10 days and most of the remaining 150 were showing signs of illness. The symptoms noted were staggering, running in circles, fever, constipation and occasionally vomition. The sows were vaccinated for hog cholera and hog cholera antiserum was ineffective as treatment.

Rectal temperatures were 106.2, 105.8 and 98.3. Total leukocyte counts on two pigs were 7500 and 27,000 with differentials of 72 and 78 neutrophils, 28 and 17 lymphocytes, one and zero myelocytes and zero and five basophils respectively. At necropsy all had petechiae in the kidneys with no other obvious gross lesions. Only a few non-specific bacteria, staphylococci, coliforms and corynebacteria were isolated from the kidneys or spleens. The CNS or other tissues were not frozen for the inoculation of rabbits or tissue cultures.

The most significant finding on histopathological examination was the diffuse non-suppurative meningo-encephalitis. Numerous intranuclear inclusions were observed in some sections of the brain, particularly the cerebrum. On this basis a diagnosis of AD was made.

The owner and his veterinarian were interviewed in March to obtain final statistics. Only 60 of the group of 220 pigs from 24 sows survived the disease. Another six sows farrowing after the epizootic began lost their entire litters and one sow had symptoms similar to those of the baby pigs but she recovered. Most of the sows were anorectic and somewhat depressed for a few days. There was a three week interval before three other sows farrowed. During this time the farrowing house was thoroughly cleaned out and disinfected. The three sows subsequently farrowed normal litters and there was no signs of disease in the pigs.

At the time of the outbreak among the young pigs a similar disease was encountered in a group of 120 pigs that averaged 70 - 80 pounds. Signs of encephalitis were noted and the mortality ranged from 45 - 55 percent.

### TABLE III

Aujeszky's Disease in Swine Herd 'T'

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Deaths/No. in Group</th>
<th>Morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing Sows</td>
<td>0/24</td>
<td>+</td>
</tr>
<tr>
<td>Baby Pigs</td>
<td>160/220</td>
<td>+</td>
</tr>
<tr>
<td>Nursing Sows</td>
<td>0/6</td>
<td>+</td>
</tr>
<tr>
<td>Baby Pigs</td>
<td>48/48</td>
<td>+</td>
</tr>
<tr>
<td>Nursing Sows</td>
<td>0/3</td>
<td>-</td>
</tr>
<tr>
<td>Baby Pigs</td>
<td>0/25</td>
<td>-</td>
</tr>
<tr>
<td>3 Months</td>
<td>50/120</td>
<td>+</td>
</tr>
<tr>
<td>5-6 Months</td>
<td>0/400</td>
<td>-</td>
</tr>
</tbody>
</table>
Another group of 400 pigs that were near market weight were in a lot well separated from the farrowing house and the group of 120. No sickness was observed in these pigs and they went to market on schedule.

On March 20, 1963, the veterinarian collected blood samples from three pigs that survived the outbreak. Two of the three had specific serum neutralizing antibodies for ADV. (Table III).

The veterinarian stated that there was a continuing problem with atrophic rhinitis in this herd. The severity depended on the particular lot of pigs and the environmental conditions.

Other livestock were present on the farm and some cattle had limited contact with the pigs but did not develop AD. One dog died during the outbreak and had rubbed or chewed much hair and skin off its back and rear quarters. This dog had opportunities to chew on carcasses of the dead pigs. There were many rodents around the farm but it was not known whether more were present immediately before the epizootic than after. The source of infection could not be established.

**Case 3: Herd 'R'**

On January 10, 1963, two live pigs, three and one-half months of age were brought to the ADDL by the owner. The pigs were from a group of seventy-five. Signs of sickness were first noticed a week previously and five pigs had died. The symptoms were vomiting, convulsions, depression, anorexia and elevated temperature. There were about 500 pigs on the farm and were divided into four principal groups - 150 three and a half to four months old, 150 five months old, 33 sows with litters in a central farrowing house and another group of 40 sows kept separately for February farrowing (Table IV). The first group of 150 pigs were divided into two groups on the basis of size and were kept in adjacent lots. The five months old group was also divided with one half being just across a fence from the previous two groups while the other half was separated from these groups by a nine foot drive. All pigs over nine weeks of age were vaccinated for hog cholera.

The two pigs submitted to the laboratory had normal temperatures. The total leukocyte counts were 20,200 and 25,500 with increased proportionate numbers of neutrophils and band cells. The gross lesions noted in both pigs were peripheral hemorrhages in the cervical lymph nodes,

<table>
<thead>
<tr>
<th>Area</th>
<th>Age Group</th>
<th>Deaths/No. in Group</th>
<th>Morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedlot</td>
<td>3.5 month</td>
<td>17/75</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4 month</td>
<td>5/75</td>
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</tr>
<tr>
<td></td>
<td>5 month</td>
<td>7/150</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Nursing Sows</td>
<td>0/33</td>
<td>+</td>
</tr>
<tr>
<td>Farrowing House</td>
<td>5-8 Week Pigs</td>
<td>100/220</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pregnant and Nursing Sows</td>
<td>0/40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Baby Pigs</td>
<td>190/320</td>
<td>+</td>
</tr>
</tbody>
</table>
pustules in the epiglottis and congested meninges. Bacteriologic examination was negative for pathogens. Histologically there was a diffuse non-suppurative meningo-encephalitis. An extract of CNS was injected subcutaneously into a rabbit and resulted in typical AD; the inoculation of tissue cultures also was positive for ADV (Table V).

**TABLE V**

<table>
<thead>
<tr>
<th>Herd 'R' - Virus Identification</th>
</tr>
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<tbody>
<tr>
<td><strong>Species Examined</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>2 pigs</td>
</tr>
<tr>
<td>2 pigs*</td>
</tr>
<tr>
<td>6 sera**</td>
</tr>
<tr>
<td>2 pigs*</td>
</tr>
<tr>
<td>5 pigs</td>
</tr>
<tr>
<td>1 dog*</td>
</tr>
</tbody>
</table>

*Dead.  
**From surviving pigs of four month age group.

Two more dead pigs were submitted to the ADDL on January 14th. The gross lesions were very similar with the exception that there were renal petechiae and extensive consolidation of the lungs. Coliforms and corynebacteria were the only bacteria isolated. Histologic findings were similar except for bronchopneumonia and hemorrhagic lymphadenitis. ADV was again isolated in a rabbit and in tissue culture.

A frozen dead dog, also brought to the ADDL on January 14, had an extensively traumatized left cheek. Histologically there was a non-suppurative encephalitis. Inoculation of a rabbit with brain suspension resulted in death in four days. This bitch had eaten portions of a pig that was necropsied on the farm. Within four days this bitch and her six pups were dead. Three sheep in a lot bordering on the lots containing the sick pigs died suddenly during the epizootic and the owner noticed that one sheep had a lacerated shoulder but thought it was due to wire cuts. It is very likely that AD also killed these sheep.

Total mortality in the three and one-half months old pigs was seventeen. Five of the 75 four months old pigs died. The disease spread to the five months old pigs with the total of seven deaths being evenly divided between the two subgroups. The symptoms observed by the owner were similar to those in the younger pigs but fewer pigs had convulsions. Some pigs, that did develop convulsions, recovered.

About three weeks after symptoms were first noted in the feeder pigs illness developed in young pigs in the farrowing house. These pigs were farrowed in December from 33 sows and mortality did not begin until just after weaning. The mortality in the 220 pigs totalled 35 percent. There were no deaths among the sows. Two dead pigs, six weeks of age, were brought to the ADDL on February 6th. Fourteen had died since losses began four days before. Congestion of the viscera, bronchopneumonia and pleuritis were the significant findings. Coliforms were the only bacteria
isolated from these pigs. Histologically there was a disseminated non-suppurative encephalitis. The inoculation of a rabbit with a CNS suspension resulted in death with symptoms and lesions of AD.

This disease was also a problem in the pigs farrowed from 40 sows in February. There was contact with the pigs of December farrowing since they were weaned in the farrowing house and some were still there when the February farrowing began. The mortality was higher in this group, approximating 60 percent. A few sows did not farrow, some farrowed dead normal sized pigs, some farrowed underdeveloped pigs, some farrowed both live and dead pigs and some farrowed normal pigs. The litters farrowed last tended to be more normal than did the earlier ones. None of the affected pigs from this farrowing were necropsied. A few sows were tested for leptospirosis with negative results.

Five pigs from the December farrowing period were brought to the ADDL on March 13. Three had marked atrophic rhinitis and middle ear infections. Histologic examination of the brains of these did show some glial nodules and focal areas of inflammation that could have been due to previous infection with ADV. All five pigs had significant titers for ADV on SN tests in tissue cultures.

Blood samples from six survivors of the four months old group were also collected and also had significant titers for ADV.

Rats were numerous on this farm but no more so than in previous years. The farrowing house was a new building and relatively rodent-proof.

**TABLE VI**

<table>
<thead>
<tr>
<th>Virus Identification in Cell Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain of Virus</td>
</tr>
<tr>
<td></td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Aujeszky</td>
</tr>
<tr>
<td>Indiana 'R'</td>
</tr>
<tr>
<td>Indiana 'S'</td>
</tr>
</tbody>
</table>

*Reciprocal of the serum dilution neutralizing 50-100 TCID50 of virus.

Table VI shows the results of cross neutralization tests using four sera and the Aujeszky,* Indiana 'S' and Indiana 'R' strains of ADV. The 'S', 'R' and 'T' sera were from surviving pigs from the three herds described herein.

**Summary of Clinical Pathology**

Rectal temperatures were taken on 15 sick pigs. Blood samples were collected from 13 pigs; total leukocyte counts were done on 13 samples, differential leukocyte counts on nine and hematocrits on seven.

*The Aujeszky strain of ADV and a specific antiserum were obtained from Dr. R. E. Shope, Rockefeller Institute for Medical Research, New York City.
The temperatures of three pigs were below 100 F, of three were 100-103 F, of six were 103-105 F and of three were 105-106.2 F.
The hematocrits of seven ranged from 38 to 48 percent.
The total leukocyte counts were as follows: one of 6650; two of 11,000-12,000; six of 15,000 to 20,000; three of 20,000 to 30,000; and one of 40,000. The percentage of neutrophils was 58 for one sample, 22 to 40 for four samples, and 71 to 86 for four samples.

Summary of Post Mortem Findings
Twenty-six swine and one dog were necropsied. Histopathologic examination of the CNS of 22 swine, one dog and five rabbits was carried out.
The gross findings in the swine were limited to meager hemorrhages in lymph nodes and renal papillae, and congestion of other viscera in some cases. In the rabbits there were abrasions at the sites of inoculation and marked congestion of the lungs and livers. The dog's face was similarly abraded about an open wound, and its viscera were congested.
The most striking microscopic lesions were present in the CNS of the swine and the dog. The lesions consisted of a diffuse, non-suppurative, panencephalomyelitis and ganglioneuritis. The lesions were characterized by perivascular cuffing, diffuse and focal gliosis, and necrosis of both neurones and glial cells (Figure 2). Type A nuclear inclusions were rare in all but one pig. In that pig inclusions were numerous in the astroglia of the cerebral white matter and less numerous elsewhere (Figure 3).

Figure 2. Perivascular cuffing, diffuse and nodular gliosis, and neuronal necrosis and phagocytosis (arrows) in a deep layer of the cerebral cortex.

Figure 3. Type A nuclear inclusion in an astrocyte of cerebral white matter.
In the swine the lesions tended to be more severe in the anterior than in the posterior regions of the brain. In the dog the lesions were limited to the brain stem. There were no CNS lesions in the rabbits.

Microscopic findings in the other viscera included congestion and edema of the lungs in several swine in the dog, and in all rabbits. Congestion of other organs was variable. Focal necrosis was seen in rabbit livers. The lymph nodes of some swine contained areas of acute necrosis associated with hemorrhage. The reticulo-endothelial cells in such nodes contained large nuclear inclusions similar to those described as associated with hog cholera.\textsuperscript{14}

DISCUSSION

The source of infection in the three herds is unknown. Rodents were numerous on the farms and could be the reservoir of infection but would not likely carry ADV from herd 'S' to herd 'T' or 'R'. Dogs could have transported the virus, as infected animals, from one herd to another since in two cases, dogs died from AD. In the case of herd 'S' possibilities of spread by personnel existed; one tractor with attached manure scoop was used on Farms A and B. There was no known interactivity of personnel attending the three herds in question. Other diagnostic laboratories in Indiana, Ohio, Illinois and Nebraska were asked if confirmed cases of AD in swine had been found during 1962 and early 1963; only one case of AD was diagnosed in mid-December 1962. The baby pigs were from another herd in the same area as the three herds reported here.

The strain of ADV involved in these outbreaks seemed unusually pathogenic for swine. Possibly a variant virus had evolved since viruses are notorious for genetic lability. Cycling of the virus within the cells of the reservoir host(s) may have resulted in mutation to high pathogenicity which dominated the virus population liberated by them. These strains in cell culture were antigenically similar to the Aujeszky strain but attained titers of $10^5$ to $10^6$ TCID\textsubscript{50}/ml. compared to $10^3$ to $10^4$ for the Aujeszky strain. Further characterization of these isolates is needed both in cell culture and in pigs. One SPF pig by contact did develop mild symptoms of AD as well as a significant SN titer.

Differential diagnosis of AD is important since it does mimic hog cholera. Some characteristics noted in this investigation should be mentioned. If associated sudden death occurs in other species such as cattle, dogs, sheep or cats, AD should be suspected. An age-resistance factor was clearly shown. Mortality was highest in newborn pigs and ranged downward to about three percent in the fat hogs and was rare in the sows. Anorexia, vomition and constipation were common symptoms with diarrhea, (as in hog cholera), being absent. Coughing and other symptoms suggestive of influenza as described by Koves and Hirt\textsuperscript{5} were noted in some older swine. Rectal temperatures of sick pigs ranged from normal to 104
or 105°F with an occasional one of 106°F. Temperatures in pigs with hog cholera or other septicemias are often higher, 107 to 108°F. After having a convulsion due to AD a pig would appear relatively normal and not have staggering gait associated with hog cholera. Treatment with anti-hog cholera serum was not effective in one herd. It would be possible for such serum to be beneficial if ADV antibodies were also present. Most of the pigs dying from AD had convulsions. Some pigs that had convulsions recovered; this is not characteristic of hog cholera.

The usual course of the disease in a herd was about seven days after the peak of morbidity had been reached with deaths being unusual after this time. At the onset of AD in the feeder pigs it was often thought that mortality would be higher than it actually was.

Leukopenia developed in some pigs in the acute phase of AD but was not as marked or as consistent as in hog cholera. Differential leukocyte counts tended to a neutrophilia rather than a neutropenia.

The gross lesions were inconsistent. Congestion of the meninges and lymph nodes was the most constant lesion. Occasionally necrotic foci in various organs, as described by Mikhailyukov, were present. Bronchopneumonia and pulmonary edema were present in some pigs. Histologically the most striking lesion was the non-suppurative panencephalomyelitis with meningitis. The distribution of lesions in the CNS did agree well with the descriptions of English workers. Intranuclear inclusions in cells of the cerebrum were present only rarely but when present are considered to be pathognomonic. A necrotizing lymphadenitis was observed in some pigs; this has not been noted as a specific lesion by other investigators. What appeared to be intranuclear inclusions in reticulum cells of lymph nodes were observed on occasion and need to be differentiated from somewhat similar inclusions reported in hog cholera.

The diagnostic test of choice was the subcutaneous inoculation of rabbits with a suspension of CNS and other tissues. Various cell culture systems were also useful in diagnosis because ADV has a wide host-cell range. This virus was found to be labile on storage at -15°C. Storage should be at refrigerator temperature (-4°C) in 50 percent glycerol-saline or below -30°C as recommended by Hungarian workers.

The serum neutralization test was of use in confirmation of ADV infection. Paired serum samples are highly desirable. This test would be useful in cases of abortions of unknown etiology. Aborted fetuses should also be examined microbiologically.

AD does appear to be a self limiting infection in a herd. Because of rapidity of spread, isolation of animals is not a practical solution. The virus is usually shed by an infected pig for two weeks. Reservoir animals and asymptomatic swine may be more important than considered previously. Two infected pigs kept in isolation did have exacerbation of clinical symptoms for a month. One had persisting microscopic foci of encephalitis five months after the acute phase.
A fatal encephalitis due to infection with Aujeszky's disease virus occurred in three swine herds in northwestern Indiana. Deaths occurred in all ages and weights of swine. Some sows aborted. Associated deaths of dogs, sheep and a cat were recorded. The highest morbidity and mortality occurred among baby pigs and the lowest among sows. Histopathologic studies were made. Other diagnostic techniques employed were animal and cell culture inoculation and serum neutralization tests.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


The Committee reaffirms the recommendations of the 1962 Committee's report and urges their adoption.

Atrophic rhinitis and virus pig pneumonia are currently recognized throughout the nation as two of the most economically serious diseases affecting the swine industry. The development of successful control programs is urgently needed. The greatest current need is the availability of atrophic rhinitis and virus pig pneumonia free breeding stock for repopulation purposes.

Because of insufficient knowledge and lack of practical diagnostic tests for certifying the freedom of swine for either of these diseases, a control program for the eradication of atrophic rhinitis or virus pig pneumonia from infected swine is not feasible at this time (it is claimed that there are many causes for atrophic rhinitis - one cause is said to be Bordetella bronchiseptica, elimination of this organism by feeding of high levels of sulfamethazine or sulfaethoxy-pyridazine in the ration is being attempted).

This Committee recommends, until such time as successful eradication methods are feasible, that the livestock disease regulatory agencies of the various states establish programs to assist the swine industry in the development of primary and secondary specific pathogen free pigs and that the United States Livestock Sanitary Association appoint a committee to establish uniform methods and standards for such programs. It also recommends that the United States Livestock Sanitary Association encourage research agencies to engage in increased investigations of successful methods to control and eradicate atrophic rhinitis and virus pig pneumonia. The Committee further recommends the following:

1. That no hypodermic injections of any kind be administered into the ham because such injections result in economic losses due to abscess formation.

2. That more attention be given to pseudorabies, which is becoming more important problem, and urges that increased attention be given to diagnosis, especially differentiation from hog cholera and the development of more effective immunizing agents.

3. That no swine in a boar testing station be permitted to be sold for breeding purposes following their performance ratings and such stations be inspected for adequate sanitation and isolation facilities while testing is underway.
GOAT SERA AS MEDIA SUPPLEMENTS IN THE EXALTATION OF NEWCASTLE DISEASE VIRUS (END) TEST FOR HOG CHOLERA VIRUS


Columbia, Missouri

INTRODUCTION AND REVIEW OF THE LITERATURE

The exaltation of the cytopathic effect of Newcastle disease virus (NDV) by hog cholera virus (HCV) in tissue culture was reported by Kumagai et al. in 1958. These investigators found that this exalting effect was a useful indicator for the laboratory detection and quantitation of HCV. The test was named the "END" method (exaltation of NDV).

As reported by Kumagai et al., NDV produced little or no cytopathic effect in primary cultures of swine testicular cells if inoculated during the first four to six days of cultivation. Newcastle disease virus inoculated after this initial period of cultivation produced cytopathic effects in these cultures. If the tissue culture cells were exposed to HCV when they were first cultivated, then NDV inoculated as early as two days later produced marked cytopathic effects in the cultures.

Matumoto et al. reported that the titers of HCV as determined by the END method were one to 1.75 \( \log_{10} \) dilutions lower than the titers of the same virus material when tested in susceptible swine. A two step method wherein HCV was passed one time in cultures of swine splenic tissue prior to testing by the END method was described. By using the two step method, virus titers comparable to those obtained by swine inoculation were demonstrated. Kumagai et al. tested 28 tissue specimens obtained from naturally and experimentally infected swine for HCV by the END method. Twenty-three of the specimens were positive while the remaining five, presumably with low virus content, were negative. Matumoto et al. reported that lapinized HCV did not exalt the cytopathic effect of NDV, presumably because of decreased ability to propagate in swine cells. These investigators also demonstrated that serum from hog cholera immune pigs completely suppressed the exalting effect of HCV, while serum from susceptible pigs did not.

In early studies, Kumagai et al. found that only four of 20 bovine serum samples were satisfactory for use in the END test. It was later reported that increasing the bovine serum concentration in the tissue culture medium from 10 percent to 20 percent increased the percentage of satisfactory serum samples to 43 percent. This paper presents the...
results of investigations into the usefulness of sera from animals other than cattle in the END test. The influence on test results of the age of the cultures and sequence of virus inoculations are also described.

MATERIALS AND METHODS

Hog Cholera Virus (HCV)

Hog cholera virus (serial number 318) was obtained through the courtesy of Doctor J. P. Torrey, National Animal Disease Laboratory, Ames, Iowa. The titer of this material was $2.5 \times 10^6$ pig infective doses per ml. when last tested by Dr. Torrey in 1960. The virus was stored at $-66\,\text{C}$. A third serial passage of this virus in cultures of swine testicular cells was used in some experiments.

Newcastle Disease Virus (NDV)

Newcastle disease virus (California 11914 (NAP)) in extraembryonic fluid was used as a stock virus preparation. The titer of this preparation was $10^{3.7}\,\text{LD}_{50}$ for egg embryos per ml. This virus was stored at $-66\,\text{C}$.

Sera

Blood was collected aseptically from cattle, sheep, and goats at the University of Missouri. The blood was allowed to clot, and the serum was removed and centrifuged to facilitate complete separation. Newborn "agamma" calf serum, specially processed to remove gamma globulin and other inhibitory factors, was purchased from Hyland Laboratories, Los Angeles, California. Pooled calf serum was purchased from Cappel Laboratories, West Chester, Pennsylvania. Pooled rabbit serum was purchased from Pel-Freeze Rabbit Meat Company, Rogers, Arkansas. Pooled sheep sera were purchased from Hyland Laboratories and from Microbiological Associates, Bethesda, Maryland. Pooled goat serum was prepared from individual sera collected and tested during the course of this study. All sera were stored below $0\,\text{C}$. Each serum was inactivated in a water bath at $56\,\text{C}$ for 30 minutes prior to use.

Glassware

Screw cap culture tubes made of borosilicate glass of low alkali content were used. These tubes were fitted with plastic caps with white rubber liners. Leighton tubes, with coverglass inserts and silicone rubber stoppers, were used for the preparation of slide cultures for photomicrographic studies.

Tissue Culture Medium

Tissue culture medium was prepared as described by Kumagai et al.\textsuperscript{1} This medium contained Hank's balanced salt solution, and 0.5 percent lactalbumin hydrolysate. Penicillin, 500 units/ml., and streptomycin,
100 μg./ml. were added. Two percent isotonic NaHCO₃ solution was added to the medium for initial cultivation of the cells. For subsequent medium changes four percent isotonic NaHCO₃ solution was added. Ten percent serum was added to the above medium.

**Tissue Cultures**

Cell cultures were prepared from testicles surgically removed from pigs two to 12 weeks old. The procedure for the preparation of the cultures was essentially that described by Kumagai *et al.*¹ with the exception that the tissues were trypsinized in 0.20 percent trypsin solution for 60 to 75 minutes at 25 °C. Cell concentrations were standardized by diluting small samples of cells in saline solution containing 0.1 percent crystal violet and counting them in a hemocytometer. The cells were diluted in culture medium so that after serum was added the final cell concentration was $2.5 \times 10^6$ cells per ml. One ml. of cells in culture medium was inoculated per tube. The cultures were incubated in stationary tissue culture tube racks at 37 °C. except as otherwise noted.

**Test Procedure**

Twelve tissue cultures were used in each test. Three cultures were not inoculated, three were inoculated with HCV only, three were inoculated with NDV only, and three were inoculated with both HCV and NDV. Except as otherwise noted, the cultures were inoculated on the day of initial cultivation (day zero) or on the following day with 0.1 ml. of a $10^{-2}$ dilution of HCV, and three or four days later with 0.1 ml. of a $10^{-2}$ dilution of NDV. In early studies on the use of bovine serum in the END test 0.1 ml. of a $10^{-2}$ dilution of blood from a healthy unvaccinated pig and of allantoic fluid from uninoculated eggs was added to appropriate control cultures. Culture medium was replaced with fresh medium two times per week or oftener prior to the final virus inoculation. Regardless of other variations, the culture medium was changed on the day before and again on the same day that the NDV was inoculated. Following the inoculation of the latter of the two viruses in each test no further medium changes were made.

**RESULTS**

*Sera from Different Animal Species in the END Test*

The results of this study are summarized in Table I. Five bovine sera were found to vary from unsatisfactory to satisfactory in their ability to support viral exaltation. The best serum was satisfactory in seven of eight trials, however, in the eighth trial incomplete cytopathic effects in dually inoculated cultures were observed. Other sera were rated as unsatisfactory because of degenerative changes in the control cultures, and incomplete or no reactions in the dually inoculated cultures. All five sera were tested two times at 10 percent and 20 percent levels in the tissue culture medium. Sera that were found to be unsatisfactory at the 10 percent level were also unsatisfactory at the 20 percent level. Incubation of the test cultures at 41 °C. did not change the results in two separate trials.
Newborn "agamma" calf serum and pooled calf serum did not support viral exaltation in dually inoculated cultures.

Individual sheep serum samples supported fair to good growth of the cells and viral exaltation was observed in dually inoculated cultures. One pool of sheep serum did not support cell growth, and another did not support viral exaltation in dually inoculated cultures. Pooled rabbit serum did not support the growth of tissue culture cells. All goat sera tested supported good cell growth. Twenty-two of thirty-one individual samples and three of three pooled samples of goat serum were found to be completely satisfactory for the END test.

A detailed summary of the use of goat serum as a medium supplement in the END test is presented in Table II. Exaltation of the cytopathic effect of NDV by HCV was marked. Extensive cytopathic effects were usually observed after 40 hours and the cultures were completely destroyed after 72 hours of incubation. With one serum sample, cytopathic effects were not observed until the 13th day. With eight goat sera, degenerative changes

### Table I

<table>
<thead>
<tr>
<th>Serum</th>
<th>Results*</th>
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</thead>
<tbody>
<tr>
<td>Bovine</td>
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</tr>
<tr>
<td>Individual samples</td>
<td>1/5</td>
</tr>
<tr>
<td>&quot;Agamma&quot; calf serum</td>
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</tr>
<tr>
<td>Pooled calf serum</td>
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</tr>
<tr>
<td>Sheep</td>
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<tr>
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<tr>
<td>Rabbit, pooled</td>
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</tr>
<tr>
<td>Goat</td>
<td></td>
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<tr>
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<tr>
<td>Pooled samples</td>
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</table>

*Number satisfactory/number tested

### Table II

<table>
<thead>
<tr>
<th>Serum</th>
<th>Number Tested</th>
<th>Results*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uninoculated</td>
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<tr>
<td>Individual samples</td>
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<td>0/93</td>
</tr>
<tr>
<td>Pooled samples</td>
<td>3</td>
<td>0/12</td>
</tr>
</tbody>
</table>

*Number of cultures with degenerative changes or cytopathic effects/number of cultures tested.

**Degenerative changes in control cultures occurred with eight of 31 serum samples tested.
occurred in some cultures other than those inoculated with both HCV and NDV. These degenerative changes were observed most often in the NDV inoculated control cultures where 14 of 24 cultures had mild to severe degenerative changes. Four cultures of twenty-four inoculated with HCV only developed similar degenerative changes. These changes consisted of rounding of the cells in isolated areas of the cell sheet. Small portions of the cell sheet became partially detached from the glass in these areas. These changes were different from the cytopathic effects observed in dually inoculated cultures in that they were usually mild and did not progress to the complete destruction of the culture. In some cases, the cultures improved upon continued incubation. When satisfactory and unsatisfactory goat sera were pooled and used in the tissue culture medium no degenerative changes were observed in the control cultures and exaltation of the cytopathic effect of NDV by HCV occurred.

Titration and Serial Passage of HCV in Swine Testicular Cell Cultures

One-tenth ml. of $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$ dilutions of HCV were tested by the END method. Cytopathic effects were observed in cultures dually inoculated with NDV and $10^{-2}$ and $10^{-3}$ dilutions of HCV. No cytopathic effects were observed with higher dilutions of HCV. Identical results were obtained in a second titration of HCV by the END method where-in the cultures were incubated at 39 C. Culture fluid from a third passage of HCV in swine testicular cell cultures was found to exalt the cytopathic effect of NDV in the END test. The dilution of the virus, considering inoculum dilutions and medium changes, was at least $10^{-3}$.

Age of Cultures and Sequence of Virus Inoculation in the END Test

The results of studies on the influence of the age of the cultures and the sequence of the inoculation of HCV and NDV on the exaltation of the cytopathic effect of NDV are presented in Table III. Goat serum was used as a medium supplement in trials one, two, and three while previously tested bovine serum was used in all other trials. Hog cholera virus inoculated on day zero exalted the cytopathic effect of NDV inoculated eight and 13 days later. The inoculation of HCV as late as the seventh day of cell cultivation followed by NDV four days later resulted in the appearance of cytopathic effects two days later in two trials. In one other trial, trial 12, the reaction was delayed and incomplete. In trial 14, cytopathic effects were observed in cultures inoculated with NDV on the fourth day followed by HCV on the 13th day of cultivation. Cytopathic effects were observed within 12 days in cultures inoculated simultaneously with both viruses on the seventh day of cultivation.

Photomicrographic Studies

Figures 1, 2, 3, and 4 are photomicrographs (x125) of an uninoculated control culture, a control culture inoculated with HCV, a control culture inoculated with NDV, and a culture dually inoculated with HCV and NDV respectively. The cultures were stained with May-Greenwald-Giemsa stain on the sixth day of cultivation. Marked cytopathic effects were
GOAT SERA IN END TEST FOR HOG CHOLERA VIRUS

TABLE III
Age of Cultures and Sequence of Virus Inoculation in the END Test

<table>
<thead>
<tr>
<th>Age of Cultures (days)</th>
<th>Inoculum</th>
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<tr>
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<td>HCV</td>
<td>NDV</td>
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<tr>
<td>1*</td>
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<td>8</td>
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<tr>
<td>2*</td>
<td>7</td>
<td>11</td>
</tr>
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<td>3*</td>
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<td>7</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>4</td>
</tr>
</tbody>
</table>

*Goat serum was used in these tests; bovine serum was used in all other tests.

**Cytopathic effects were observed in one of three dually inoculated cultures.

observed in cultures dually inoculated with HCV and NDV. The cells in these dually inoculated cultures condensed into multinucleated masses which later detached from the inner surface of the culture tube.

DISCUSSION

These studies confirm the findings of Kumagai et al. 2 of an exalting effect of HCV on NDV infections in primary cultures of swine testicular

Figure 1. Uninoculated Culture of Swine Testicular Cells (x125)
Figure 2. Culture of Swine Testicular Cells Inoculated with HCV (x125)

Figure 3. Culture of Swine Testicular Cells Inoculated with NDV (x125)

cells. The observation that one of five bovine serum samples was satisfactory for the test is in agreement with the findings of Kumagai et al. wherein 20 percent to 43 percent of all bovine sera were satisfactory for the END test.\textsuperscript{1,2} Increasing the concentration of the bovine serum in the tissue culture medium from 10 percent to 20 percent did not appreciably change the results in this study. Increasing the temperature of incubation
of the cultures to 41 C. also did not materially change the results. At 41 C. the tissue culture cells appeared smaller than cells in comparable cultures incubated at 37 C. The cells also appeared to be more granular when incubated at the higher temperature. Some bovine sera found to be generally unsatisfactory in the test occasionally gave entirely satisfactory results, however, on repeated testing the reactions were inconsistent.

Evidence that this test is an exaltation of NDV by HCV and is not caused by other constituents of the virus inoculum was obtained with HCV which had been serially passed in swine testicular cells three times. This virus retained its ability to exalt the cytopathic effect of NDV. The dilution of the HCV inoculum in this experiment tends to rule out extraneous factors in the inoculum as those which exalt the NDV infection. Similar observations with tissue culture propagated HCV have been reported by Kumagai et al.1

As little as 0.1 ml. of a $10^{-3}$ dilution of HCV exalted the NDV infection in tissue culture. This inoculum represents 250 pig infective doses of HCV. Matumoto et al.3 reported that the direct test, as performed here, was sensitive to as few as 10 to 56 pig infective doses of HCV. This difference in sensitivity is quite likely due to differences in the bovine serum used. While the most satisfactory bovine serum available was used for this titration, it gave a slower reaction to the END test than did some other sera tested. It appears from this study, and from the successful END tests using HCV serially passed in tissue culture, that the test ultimately will be restricted in sensitivity only by the susceptibility of primary swine testicular cell cultures to HCV. Other factors, such as adequate time for virus propagation and adequate pH control during long term virus propagation are technical problems that can be readily solved.
Goat serum in general was found to be much superior to bovine serum as a medium supplement in the test. The use of goat serum resulted in a more consistent and rapid response on the part of the cells to the synergistic cytopathogenicity of HCV and NDV. The cytopathic effect involved the entire culture and the destructive processes progressed rapidly to completion, hence the reading of endpoints was greatly facilitated. The only difficulty encountered in using goat serum for the test was the occasional occurrence of degenerative changes in control cultures which occurred with eight of 31 serum samples tested. These changes typically did not progress to the total destruction of the culture, and in most instances these degenerative changes could be readily distinguished from the cytopathic effects observed in dually inoculated cultures. When goat sera that gave rise to degenerative changes in the control cultures were pooled with other goat sera and used in the END test, no degenerative changes in control cultures were produced.

The time of introduction of NDV into the cultures has been reported as a critical factor in the use of the END test. Kumagai, et al.\textsuperscript{2} reported that the successful use of the test required the inoculation of NDV into the HCV infected cultures prior to the fifth day of cell cultivation. They further reported that the entire test should be completed within seven days because of normal degenerative processes which appeared after eight to twelve days of cultivation in all of the cultures. In the studies reported here, the time of inoculation of NDV was not found to be a critical factor. Newcastle disease virus inoculated as late as the 13th day produced cytopathic effects in HCV infected cultures but not in control cultures. Cultures inoculated with HCV after seven days of cultivation and NDV four days later developed cytopathic effects of the dual virus infection. Some irregularities in the results were observed with older cultures when bovine serum was used as the medium supplement. This was not surprising since it was found that with all bovine sera tested occasional irregularities did occur for which there was no explanation. When goat serum was used as a medium supplement irregularities were reduced to a very low degree with young cultures. With older cultures no irregularities have been observed in the test in the limited studies undertaken so far. The observation in one trial that NDV could be inoculated prior to HCV requires further study. The inoculation of NDV either before or simultaneously with HCV did not reduce the total time required for the test, in fact, it sometimes took longer for cytopathic effects to become visible.

The normal degenerative process described by Kumagai et al.\textsuperscript{1,2} as occurring in cultures on the eighth to the twelfth day of cultivation was not observed in this study until after the 21st day if the pH and nutritional content of the medium was satisfactory. The successful long term maintenance of primary cultures of swine testicular cells reported here may be due to more suitable glassware, glassware washing procedures, or culture tube stoppering devices. It has been observed in this laboratory that silicone rubber stoppers readily absorb chemicals, including disinfectants, which may cause degeneration of the cells after variable periods of incubation.
The longer useful life of the cultures in this study greatly increases the value of the test for the detection of the presence of HCV. Monolayer cultures may be prepared and maintained for later use if the pH of the culture medium is properly controlled and if the medium is replaced at regular intervals. This modified technique also permits the inoculation of large amounts of material suspected of containing small amounts of virus. After allowing the HCV to attach to susceptible cells the toxic substances of the inoculum may be removed and the cultures rinsed and replenished with fresh medium. Thus, the END method provides a practical means for the isolation of HCV and a suitable indicator system for a sensitive serum neutralization test.

**SUMMARY**

Sera of cattle, sheep, rabbits, and goats were tested as medium supplements in the exaltation of Newcastle disease virus (END) test for hog cholera virus. Goat serum was the best medium supplement. Twenty-two of thirty-one individual goat serum samples were completely satisfactory for use in the test. Nine of the thirty-one serum samples were unsatisfactory because of the appearance of degenerative changes in control cultures or, in one instance, because of the delayed appearance of cytopathic effects in dually inoculated cultures. Three of three pooled goat serum samples were satisfactory for use in the test.

Positive END tests were obtained with 250 or less pig infected doses of hog cholera virus. Primary cultures of swine testicular cells were satisfactory for the test from zero to seven days of age. Cultures were maintained in good condition for 21 days.

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr. H. C. McDougle for preparing the photomicrographs. Appreciation is also expressed to Dr. D. E. Rodabaugh for making swine tissues available for this investigation.

**REFERENCES**


DEVELOPMENT OF HETEROTYPIC BOVINE VIRUS DIARRHEA (BVD) VACCINE AGAINST HOG CHOLERA

Peter H. Langer, V.M.D., Ph.D.*
Kansas City, Missouri

Recent studies have shown that pigs vaccinated with bovine virus diarrhea (BVD) virus survived challenge with hog cholera (HC) virus that killed unvaccinated controls. It was also noted that the degree of protection correlated with the strain of HC virus used for challenge.

Because of the favorable outcome of some of these preliminary tests and the potential usefulness of this vaccine in an eradication program, the development of a BVD vaccine was initiated at our research laboratory. Furthermore, experiments were designed to replicate reported results; to compare the efficacy of BVD vaccine, rabbit origin HC vaccine and crystal violet vaccine; and to study the effects of different strains of HC challenge viruses utilizing both randomly selected as well as littermate pigs in these tests.

MATERIALS AND METHODS

Preparation and Testing of BVD Vaccine. The seed virus used in the preparation of this vaccine was obtained through the courtesy of Dr. James A. Baker, Veterinary Virus Research Institute, Cornell University. It was received from frozen spleen obtained from a disease-free calf which had been infected with BVD virus, Strain NY-1. A 10 percent emulsion of this splenic material was inoculated into tubes containing primary bovine embryo kidney tissue cultured cells maintained with a medium consisting of Earle's balanced salt solution, lactalbumin hydrolysate and two percent horse serum. These cultures were harvested on the fifth day and served as the inoculum for the production of second tissue culture passaged virus in Povitsky bottles. This material was harvested on the fourth day, combined with equal parts of stabilizer-extender and lyophilized in five-dose containers. The titer of this vaccine, designated as Serial No. 1, was $10^{4.7}$ per ml. and thus contained 100,000 TCID$_{50}$'s per two ml. dose.

Titration of the vaccine was performed by the cellular interference method since BVD virus, Strain NY-1, is noncytopathogenic.

A pig potency test was conducted on BVD Serial No. 1. Fifteen pigs, which were of the same weight, age and source as those utilized for routine HC vaccine potency tests, were purchased. Ten pigs were randomly selected and vaccinated intramuscularly with two ml. of vaccine, while the remaining five pigs served as contact controls. Two weeks after vaccination, all pigs were transferred to the regular HC challenge area and challenged with virulent Sioux Falls strain HC virus.

*From the Biological Research Department, Jensen-Salsbery Laboratories, Division of Richardson-Merrell Inc.
TABLE I
Response of Pigs Vaccinated with BVD Vaccine* Followed by Challenge with HC Viruses

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Age</th>
<th>Challenge</th>
<th>Challenge Virus</th>
<th>Challenge Route**</th>
<th>No. Pigs Surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency Test Pigs</td>
<td>Vaccination</td>
<td>Challenge</td>
<td></td>
<td></td>
<td>Vaccinates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 1/2 mos.</td>
<td>3 mos.</td>
<td>Sioux Falls</td>
<td>S.C.</td>
</tr>
<tr>
<td>Field Test Pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm #4 - Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 1/2-3 mos.</td>
<td>4 mos.</td>
<td>Sioux Falls</td>
<td>I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 1/2-3 mos.</td>
<td>4 mos.</td>
<td>Sioux Falls</td>
<td>Cont.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 1/2-3 mos.</td>
<td>8 mos.</td>
<td>Sioux Falls</td>
<td>L.M.</td>
</tr>
<tr>
<td>Farm #7 - Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2 1/2 mos.</td>
<td>6 mos.</td>
<td>Sioux Falls</td>
<td>I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2 1/2 mos.</td>
<td>6 mos.</td>
<td>Ames (318)</td>
<td>I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2 1/2 mos.</td>
<td>6 mos.</td>
<td>Strain A</td>
<td>I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2 1/2 mos.</td>
<td>7 mos.</td>
<td>Sioux Falls</td>
<td>L.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2 1/2 mos.</td>
<td>10 mos.</td>
<td>Sioux Falls</td>
<td>Cont.</td>
</tr>
</tbody>
</table>

*Jen-Sal Serial No. 1, titer 104.7/ml., administered intramuscularly as a 2 ml. dose.
**S.C. = subcutaneous
I.M. = intramuscular
Cont. = contact exposure

The results of this potency test are tabulated in Table I. No clinical signs of illness were observed in the vaccinated pigs. There was a typical clinical HC response and lesions indicative of HC were seen on necropsy in the control pigs.

Serologic Test. The BVD serum-neutralization test was performed according to the method previously described.4

FIELD TRIALS

Studies were initiated to determine if BVD vaccine, Serial No. 1, was at least 90 percent efficacious under field conditions in protecting pigs eight weeks of age or older against hog cholera.

Vaccination Procedure. Due to the lateness of the season, which resulted in a limited availability of pigs of suitable age, only three farms could be selected for this field trial. Since littermates could no longer be identified, the vaccinated and control pigs designated for challenge were selected by a random number system and identified by ear notching. Approximately 900 pigs were eventually vaccinated on these three farms. Blood samples were taken at the time of vaccination, prior to challenge and seven to 14 days after challenge in those surviving. The animals from one of the three farms could not be challenged due to the inadvertent marketing of the pigs.

General husbandry practices on Farm No. 4 were fair. The pigs were of mixed breeding, predominately Yorkshire. Most appeared thrifty, although some showed clinical evidence of pneumonia and a heavy louse infestation was noted in some of the younger pigs.

Farm No. 7 had average to good management. The pigs were of Hampshire-Duroc breeding. Most appeared thrifty, although a heavy louse
infestation was present. Erysipelas bacterin was administered simultaneously with the BVD vaccine. Castrations were performed on all farms at time of vaccination.

In order to determine if protection lasted until pigs were of market age, the majority were challenged when six to 10 months old.

Three strains of HC challenge viruses were used: Sioux Falls; Ames, Serial 318, furnished through the courtesy of Dr. G. O. Johnson, NADL, Ames, Iowa; and Strain A furnished through the courtesy of Dr. J. A. Baker.

Two groups of pigs were challenged by contact exposure.

Results. The results are tabulated in Table I. Although the over-all efficacy of the pigs challenged with Sioux Falls and Ames strains in all groups was poor (48 percent), it did demonstrate that some pigs were protected up to 10 months of age or eight months post-vaccination. Pigs in Groups 1 and 2 from Farm No. 4 had shown evidence of pneumonia at the time of vaccination and very extensive pneumonia at the time of challenge. This was confirmed on necropsy. Group 3, Farm No. 4, challenged at eight months of age had no clinical evidence of pneumonia and had a better response to challenge. No pneumonia was seen on necropsy in the one animal that died in this group. Five out of eight pigs in Group 5, Farm No. 7, survived contact challenge with no adverse clinical reactions.

All pigs challenged with Strain A HC virus in Group 3, Farm No. 7, had excellent response. They remained active, on full feed and had a fever lasting but one to three days.

The pigs in Group 3, Farm No. 4, and Groups 4 and 5, Farm No. 7, were animals which had been vaccinated but not further identified. At the time they were brought in for challenge, no unvaccinated control pigs remained on these farms since they had been used for previous tests or sold.

COMPARATIVE STUDIES

Since the Missouri field tests failed to confirm the results of the potency test, further field trials were discontinued. The Florida field trials and the results in pigs from Farm No. 7 have shown that pigs vaccinated with BVD vaccine and challenged with Strain A HC virus had almost 100 percent protection with no adverse clinical effects. Although the results obtained with Strain A HC challenge virus were encouraging, conventional HC vaccines must pass potency test requirements by withstanding challenge with virulent strains of virus represented by Strain "Sioux Falls" and Strain "Ames." In view of these current requirements, comparative vaccine studies were started in littermate pigs.

The pigs from the Missouri trials that survived challenge with Sioux Falls or Ames HC viruses did so with little or no adverse clinical response. The majority of the pigs that succumbed presented clinical and pathologic evidence of pneumonia. It was felt that, if clinically healthy subjects were vaccinated and secondary infections controlled, the response to challenge would improve. Therefore, the following tests were performed:
**Test No. 1** - Six litters, seven to nine weeks of age, were vaccinated with BVD and rabbit origin vaccine (ROV*). In each litter, two pigs received 50,000 TCID$_{50}$ of BVD vaccine intramuscularly; two pigs received ROV intramuscularly plus 15 ml. serum and were held in separate quarters for two weeks after vaccination after which time all pigs were housed in a common area; and one pig was retained as a contact control with the BVD vaccinated pigs.

All pigs were challenged by contact exposure in the hog cholera challenge area with Sioux Falls HC virus when approximately five months of age.

In order to obtain a suitable supply of test animals representative of Midwestern swine husbandry practices, a farm (McCaughey) was located on the outskirts of Kansas City. This farm offered relative isolation from other swine producers, control over vaccination, general husbandry and breeding procedures, and the opportunity to select litters varying in age and size.

**Test No. 2** - Prior to the time that the results from Test No. 1 were available, four McCaughey litters, six to eight weeks of age, were vaccinated in the following manner: Two to five pigs in each litter received 50,000 TCID$_{50}$ of BVD vaccine intramuscularly; two pigs received ROV plus 15 ml. serum intramuscularly; two pigs received crystal violet (CV**) vaccine intramuscularly; and two pigs served as contact controls. All pigs were vaccinated at the same time and housed together until challenged when approximately six months of age by contact with simultaneous HC virus in the HC challenge area.

**Test No. 3** - Four McCaughey litters of preweaning age (four to five weeks) were vaccinated in the following manner: Four pigs received 50,000 TCID$_{50}$ BVD vaccine intramuscularly; two pigs received CV vaccine; and four pigs served as controls. At 3-1/2 months of age all pigs were placed in isolation units. Two litters were challenged with Strain Andrade, one litter with Strain Ames (319) and one litter with simultaneous virus inoculated intramuscularly in each instance.

Current serials of ROV and CV vaccines which had passed ARS potency requirements were used in these tests.

**Results.** The results of these three tests are tabulated in Table II.

In Test No. 1, four out of 12 BVD, 11 out of 11 ROV vaccinated pigs and one control pig survived challenge. On necropsy, neither the control nor the BVD vaccinated pigs had any evidence of secondary infection, but did present pathologic changes indicative of hog cholera.

In Test No. 2, two out of 13 BVD, seven out of seven ROV, and four out of eight CV vaccinated pigs survived challenge. All control pigs died. Two of the surviving CV pigs had a prolonged clinical illness characterized by fever for 11 and 15 days, inappetence, and marked lethargy. No signs of secondary infection were noted clinically or on necropsy in those that died.

Test No. 3 indicated that eight out of eight BVD vaccinated pigs survived challenge with Strain Andrade with a minimum of adverse clinical

*ROV brand of hog cholera vaccine, modified live virus, rabbit origin supplied by Jensen-Salsbery Laboratories.

**Crystal violet vaccine supplied by Jensen-Salsbery Laboratories.
TABLE II
Comparative Response of Pigs Vaccinated with BVD, ROV and CV Vaccines Followed by Challenge with Various Strains of HC Virus

Test 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Pigs Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Litter No. 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HC Virus SF*</td>
<td>SF</td>
</tr>
<tr>
<td>BVD</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>ROV</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Cont.</td>
<td>1/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Test 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Pigs Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Litter No. 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HC Virus SV**</td>
<td>SV</td>
</tr>
<tr>
<td>BVD</td>
<td>0/2</td>
<td>1/3</td>
</tr>
<tr>
<td>ROV</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>CV</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Cont.</td>
<td>0/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

Test 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Pigs Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Litter No. 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HC Virus Andrade†</td>
<td>Andrade†</td>
</tr>
<tr>
<td>BVD</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>CV</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Cont.</td>
<td>1/3</td>
<td>2/4</td>
</tr>
</tbody>
</table>

*Sioux Falls Strain
**Simultaneous Virus
†Hawaiian isolate No. 2
††Strain Ames - Serial 319

response; one out of three survived simultaneous virus challenge (one pig died from injury prior to challenge); and one out of four survived Ames challenge. All CV vaccinated pigs survived, although the four pigs challenged with simultaneous and Ames viruses showed marked illness characterized by prolonged fever, inappetence and lethargy.

Eight out of the total of 18 ROV vaccinated pigs had a post-challenge temperature response of greater than 104 F for two to six days. However, none had any visible signs of illness and remained on full feed. It was also
DEVELOPMENT OF BVD VACCINE

noted that challenge by contact exposure was equivalent to challenge by a parenteral route, although the average time of death was occasionally extended by three to four days in both vaccinated and control pigs.

**Serologic Results.** BVD serum-neutralization tests indicate that 98 percent of the pigs vaccinated intramuscularly have a primary BVD antibody response with a titer of 1:2 to greater than 1:40. One hundred percent of the pigs that survived challenge had a secondary rise in BVD antibodies when screened at a 1:500 dilution. In addition, 50 percent of the pigs that died, in which a bleeding could be taken between the seventh and 14th post-challenge day, had a secondary BVD antibody response.

**DISCUSSION**

The use of a heterotypic vaccine for the protection of animals against homotypic viruses is a revolutionary concept. The use of heterotypic BVD virus for protecting pigs against HC is one example of this new approach for the control of animal diseases. Because of the great potential this vaccine offers as an important tool in the eradication of HC, it deserves careful, step by step development and evaluation. Several important problems have been identified by both the Cornell studies as well as our own. A principal question involves the replicability of the challenge response.

The potency test described above demonstrated that pigs were fully protected even when challenged with the same HC virus used in potency testing conventional HC vaccines. Yet when pigs which were vaccinated on the farm were challenged in the same manner, the results were erratic. The fact that some pigs survived challenge with no adverse clinical response eight months after vaccination would indicate that the difference in response was not related to time.

A second closely related problem concerns the degree of protection observed when various strains of HC virus were used in challenging these pigs. The data presented in Table III demonstrates this clearly.

These strains of HC viruses have arbitrarily been placed in three groups. The first has been designated "industrial viruses" and consists of those strains in which virulence has been maintained by pig to pig passage for use as simultaneous virus, inoculating virus or potency test challenge virus. Strain A has been placed in a separate group since the purity and virulence of this virus has been maintained by passage through SPF pigs. The third group consists of recently isolated field strains. Schwarte has found that the acute, septicemic forms of HC are less prevalent today, while the chronic, low grade infections appear to be increasing. Studies conducted with recently isolated strains confirm the existence of a more chronic type of HC in various geographical areas of the United States today. Of the eight isolations made, none were of the type represented by such strains as "Ames" or "Sioux Falls."

In view of these findings and to prove whether or not BVD vaccine is the antigen of choice in the HC eradication program, field trials should be organized. These must be well planned and well controlled so that the information derived will be valid and meaningful. Furthermore, these trials
<table>
<thead>
<tr>
<th>Strain (HC)</th>
<th>Lab.</th>
<th>Treatment**</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial Viruses†</td>
<td>Jen-Sal Cornell</td>
<td>37/79 46% 8/12 66% NT</td>
<td>2/43 4%</td>
</tr>
<tr>
<td>Strain Jen-Sal Cornell</td>
<td>27/60 NT 18/18 100%</td>
<td>0/25 3%</td>
<td></td>
</tr>
<tr>
<td>Recent Jen-Sal‡ Isolates Cornell(†)</td>
<td>5/5 97% NT NT</td>
<td>3/7 16%</td>
<td></td>
</tr>
<tr>
<td>Recent Jen-Sal‡ Isolates Cornell(†)</td>
<td>68/70 NT NT</td>
<td>1/32</td>
<td></td>
</tr>
<tr>
<td>Recent Jen-Sal‡ Isolates Cornell(†)</td>
<td>8/8 98% 4/4 96% NT</td>
<td>3/24</td>
<td></td>
</tr>
</tbody>
</table>

*Cornell data - courtesy of Dr. James A. Baker.

**Denominator = number challenged; Numerator = number surviving.
†Sioux Falls, Ames (Serials 318, 319) and other simultaneous virus strains.
‡Strain Andrade (Hawaiian Isolate No. 2).
(†)Strains Georgia I and II, Illinois I and II, Kansas, Hawaii and Andrade.
NT = Not Tested.

should be conducted in various geographical areas to afford adequate challenge with a variety of strains of HC under all conditions of swine husbandry. It is suggested that such field studies be conducted for at least one year.

It was observed that 98 percent of the pigs vaccinated with BVD vaccine had a primary antibody response. This indicates that BVD virus is a good antigen on the basis of serologic conversion. Although all pigs which survived had a secondary antibody response, it was puzzling to note that approximately half of the pigs that died also had a similar response. Since this secondary antibody response involves a rise of both BVD and HC antibodies, no explanation can be offered at this time as to why these pigs died of HC.

Additional studies are in progress which show promise in solving some of the variables observed in the use of this heterotypic vaccine.

**SUMMARY**

A tissue cultured BVD vaccine has been produced and tested in pigs under field conditions. Several strains of HC viruses were used to challenge some of these vaccinated animals. Although 98 percent of the pigs had a primary BVD antibody response, the degree of protection noted by survival or death varied from zero to 100 percent and appeared to correlate with the strain of HC challenge virus used. However, clarification is necessary of other important variables affecting efficacy of the vaccine and further vaccine developmental studies and field trials are needed before the full potential of this heterotypic vaccine can be realized.
ACKNOWLEDGMENTS

The author is grateful to Dr. L. A. Rosner, State Veterinarian of Missouri, Dr. H. P. Callaway, practitioner, Waverly, Missouri, and Dr. Stanley Vazey for their assistance in the Missouri field trial. The author is also grateful to Mary Jo Martin and Jerry Volenec for their technical assistance.

REFERENCES


POSSIBILITY OF HOG CHOLERA ERADICATION WITH BVD VACCINE†

James A. Baker*, Leroy Coggins*, Douglas Robson**, and Ben Sheffy*

Eradication of hog cholera means not only suppression of clinical illness but the obliteration of all living hog cholera virus, whether it be attenuated or virulent. The transmission of natural hog cholera virus must be interrupted. The use of live hog cholera virus vaccines must be discontinued. If regulatory practices could achieve this without the use of any biological products, then a swine population could result, free of hog cholera, but, of course, fully susceptible to hog cholera. In theory, this might be done by tremendous cooperative efforts verging on the magical: all living swine could be slaughtered, all meat and garbage sterilized, and an entirely new, disease-free swine population introduced by surgical procedures. All possible reservoirs of further infection would have to be destroyed. Obviously, such methods could not easily be followed in a large country such as the United States, even if the results to be obtained were completely desirable, that of securing a new swine population that although free of hog cholera is susceptible.

There is now, however, for the first time, promise of success in the great goal of complete eradication of hog cholera from the United States. As such a campaign proceeds, the swine population can be kept fully protected against natural hog cholera by vaccines that do not contain any live hog cholera virus. At the present time two products could serve this purpose: crystal violet vaccine or heterotypic BVD vaccine. In order to determine the effectiveness of these two vaccines in protecting pigs known to be susceptible to hog cholera, and, in addition, to assess the virulence of field strains of hog cholera, as it is appearing at the present time, strains of natural hog cholera virus from various sections of the continental United States, and from the State of Hawaii, have been collected through the cooperation of The Animal Disease Eradication Division of the Agricultural Research Service, as will be mentioned in more detail later. These various strains of hog cholera virus have been sent to the Veterinary Virus Research Institute for tests. They were inoculated into unvaccinated susceptible pigs as controls and into other pigs that had been vaccinated either with crystal violet or with BVD vaccine. The results of these tests follow.

Test Pigs: From New York's Newark State Farm, pigs were used that repeatedly had shown poor efficacy against Ames challenge after prior vaccination with BVD. This was done in order to reveal whether or not

†This paper was supported by a grant from the Office of Naval Research.
*From Veterinary Virus Research Institute
**From the Biometrics Unit, Cornell University
any field material appeared to be exactly like the Ames challenge. In this particular test, part of a litter of pigs was vaccinated while others were retained as controls.

**Vaccination:** Crystal violet (CV) vaccine prepared by the Colorado Serum Company and furnished through the courtesy of Mr. Majon Huff, was used, and at least three pigs of a litter were vaccinated according to direction; that is, five ml. of vaccine were given subcutaneously to each pig. Another part of the same litter was vaccinated with BVD vaccine and each pig received intramuscularly more than 1,000 tissue cultured titrated doses of vaccine, or 100 times the 50 percent protection dose. Remaining pigs in the litter were retained unvaccinated. All pigs were bled at the time of vaccination, at the time of challenge, and 14 days after challenge if they survived.

**Challenge Virus:** Under a directive from Drs. Francis Mulhern, Gilbert Wise, and Milton Tillery to field representatives of the Animal Disease Eradication Division of the Agricultural Research Service, the request was made that "The specimens must be collected from a herd in which no swine have been vaccinated against hog cholera. The virus must be harvested from one hog typically ill with hog cholera in a herd in which a field diagnosis of hog cholera is made on the basis of history, symptoms, and typical post mortem lesions. Specimens collected for this purpose must include a minimum of 30 cc's whole blood or a whole spleen." Such specimens were to be shipped to the Veterinary Virus Research Institute for inoculation into test pigs. Specimens thus far received and tested:

<table>
<thead>
<tr>
<th>Herd Identification</th>
<th>Address</th>
<th>Veterinarian*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellis Doyle</td>
<td>Sylvania, Georgia</td>
<td>Dr. R. T. Rogers &amp; J. H. Dunn</td>
</tr>
<tr>
<td>O. E. Dickerson</td>
<td>McLeansboro, Illinois</td>
<td>Dr. I. C. Bunn</td>
</tr>
<tr>
<td>Charles Butts</td>
<td>RD #1 Oswego, Kansas</td>
<td>Dr. S. Lester Jackson</td>
</tr>
<tr>
<td>Garrett Cornwell</td>
<td>W. Union, Illinois</td>
<td>Dr. Gerald C. Richardson</td>
</tr>
<tr>
<td>J. W. Brannen</td>
<td>Rt. 5 Statesboro, Georgia</td>
<td>Dr. R. T. Rogers</td>
</tr>
<tr>
<td>Andrade**</td>
<td>Hawaii, Hawaii</td>
<td>Dr. Chase Folger</td>
</tr>
</tbody>
</table>

*To these ADE field personnel for their aid in this important study, our grateful appreciation for their excellent cooperation.

**This Hawaiian virus was included because it came from an Island on which no vaccination had been practiced and pigs undoubtedly had become infected, through the feeding of uncooked military garbage, with a strain of hog cholera that had originated in the continental USA.

Materials from the natural hog cholera outbreaks were received either as spleen or defibrinated blood except that from Hawaii came as brain material which was inoculated into a pig whose blood, harvested seven days later, was used as an inoculum. Each pig was inoculated into the axillary space either with two ml. of 10 percent spleen suspension or two ml. of defibrinated blood.

**Evaluation:** Temperatures were taken daily and recorded. The appetite was scored according to the method used by the Agricultural Research Service in their Florida work, as follows:
Appetite Points X 2 = Percent = Score*

\[
\begin{array}{ccc}
X & 0 & = 0 \\
\otimes & 1 & = 2 \\
\otimes & 2 & = 4 \\
\otimes & 3 & = 6 \\
\end{array}
\]

*100 minus percent gave the score. A score of 80 or greater was considered protection.

In addition to daily temperatures and appetite score, antibody response to vaccination was determined for each pig. Most important, pigs at the time of challenge were shown to be devoid of hog cholera antibodies in order to have a valid test. Development of hog cholera antibodies after test in surviving pigs proved challenge material contained hog cholera virus.

**Conclusions:** All strains except O. E. Dickerson, McLeansboro, Illinois, killed controls. Average clinical findings were: elevated temperatures fourth day, food intake impairment eight to 10 days, and death about 20 days after inoculation; in contrast to the Ames challenge, which produced elevated temperatures one day, food intake impairment two to three days, and death eight to 10 days after inoculation; and to strain A virus, which produced elevated temperatures two days, food intake impairment five days, and death 10-12 days after inoculation (Table I). These studies showed natural hog cholera virus was not as virulent as some of the strains of hog cholera virus that have been maintained artificially for test purposes. Many workers would agree that past distribution of virulent virus for vaccination has resulted in an erroneous impression of natural hog cholera in this country. Reports indicated that natural hog cholera virus was less virulent in countries such as Japan and Australia, where the mortality rate from hog cholera was low and where vaccination that employed virulent virus has not been practiced. In 1961 Schwarte reported that natural hog cholera in the United States was less virulent than had been thought, and more closely resembled that of Australia and Japan.

BVD vaccine protected all of the pigs except one that were given field strains of hog cholera and protected equally as well, or perhaps even better, than crystal violet vaccine which failed to protect two pigs (Table II). The pig given BVD vaccine and one of the two given crystal violet

### Table I

Comparison of Signs of Illness Between Field Strains, Ames, and Strain A Hog Cholera Virus

<table>
<thead>
<tr>
<th>Days After Inoculation</th>
<th>Elevated Temp.</th>
<th>Impaired Appetite</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames</td>
<td>1-2</td>
<td>2-3</td>
<td>8-10</td>
</tr>
<tr>
<td>A</td>
<td>2-3</td>
<td>6-7</td>
<td>10-12</td>
</tr>
<tr>
<td>Field Strains</td>
<td>4-5</td>
<td>8-10</td>
<td>17-22</td>
</tr>
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</table>
TABLE II
Response of Swine to Field Strains of Hog Cholera Virus

<table>
<thead>
<tr>
<th>Field Strain</th>
<th>BVD</th>
<th>CV</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doyle-Ga.</td>
<td>7/7</td>
<td>5/6</td>
<td>0/4</td>
</tr>
<tr>
<td>Dickerson-Ill.</td>
<td>4/4</td>
<td>3/3</td>
<td>2/2**</td>
</tr>
<tr>
<td>Butts-Kansas (a)</td>
<td>2/3</td>
<td>2/3</td>
<td>0/2</td>
</tr>
<tr>
<td>(b)</td>
<td>4/4</td>
<td>3/3</td>
<td>0/1</td>
</tr>
<tr>
<td>Cornwell-Ill.</td>
<td>4/4</td>
<td>3/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Brannen-Ga.</td>
<td>3/3</td>
<td>3/3</td>
<td>0/1</td>
</tr>
<tr>
<td>Andrade-Hawaii</td>
<td>7/7</td>
<td>6/6</td>
<td>0/4</td>
</tr>
<tr>
<td>Total</td>
<td>31/32</td>
<td>25/27</td>
<td>2/16</td>
</tr>
</tbody>
</table>

*Denominator indicates pig used. Numerator indicates pigs that scored with acceptable protection.

**Note Dickerson-Ill. did not produce sufficient signs of illness for evaluation of vaccines.

Vaccine were not protected when given strain Charles Butts, Oswego, Kansas, as it came from the field. This material was heavily contaminated with bacteria. However, when blood from the two inoculated control pigs was obtained seven days after inoculation and then used for challenge, all of the BVD and CV vaccinated pigs were protected perfectly.

The six field strains tested showed no more virulence than strain A against BVD vaccine protection (and presumably these six represented the most severe cases occurring during the four month period of April through August in the alerted states). Ignoring the parenthetic fact, we could assert with 95 percent confidence that less than 40 percent of field outbreaks during this period were equal in virulence to the Ames challenge. Acknowledging the parenthetic fact that several hundred outbreaks must have been observed and found to be so mild that a hog cholera diagnosis was uncertain, we must then conclude that the proportion of Ames-type strains in field outbreaks could only have been extremely small to escape detection during this period.

Because BVD vaccine has the following characteristics, it could be used in a campaign to eradicate hog cholera from the United States:

1. BVD vaccine produces excellent protection against field strains of hog cholera virus.
2. BVD vaccine virus has been shown to be safe, because it does not spread from vaccinated pigs to other pigs nor does it spread from vaccinated pigs to susceptible cattle kept in close contact.
3. Any amount of BVD vaccine can be made readily, because it is a tissue cultured product.
4. Bovine embryos are used for tissue culture of BVD virus, a tissue culture system thought to be free of any contaminating viruses that might be pathogenic for pigs.
5. A TCID$_{50}$ of 30 protects a pig against hog cholera virus. BVD vaccine that contains 3000 TCID$_{50}$ provides 100 times the 50 percent protective dose. BVD vaccine, therefore, can be standardized by laboratory assay for assured efficacy.

6. Hog cholera antibody from maternal colostrum or from any other source does not interfere with efficacy of BVD vaccine; hog cholera antiserum can be given before, during, or after BVD vaccination if this antiserum is free of BVD antibody.

7. Because it is not hog cholera virus and does not contain any hog cholera virus, BVD virus cannot be responsible for any hog cholera outbreak.
Killed virus vaccines for immunization of swine against hog cholera were developed almost concurrently with the live virus and serum vaccination technique but most research efforts were directed toward improving the latter method of vaccination. McBryde and Cole state that crystal violet was first used for attenuation of hog cholera virus by Dorset in 1934. Laboratory experiments and field tests proved that this method was very effective in producing a stable, potent vaccine. The use of glycerol to control contamination in crystal violet vaccines further improved their effectiveness. Killed virus vaccines could be used for immunizing pregnant sows and swine in poor health without unwanted side effects. However, these vaccines could not be used effectively with anti-hog cholera serum and they were ineffective in producing a lasting immunity in pigs possessing maternal hog cholera antibodies. These properties plus the time required to develop immunity after vaccination limit the practicality of killed virus vaccines in an enzootic hog cholera area.

Cole, Torrey, and Zinober reported on the successful use of crystal violet glycerol vaccine for a 12-year period in Iowa. Research on vaccination, using two doses of crystal violet hog cholera vaccine not less than four weeks apart, was done utilizing the Animal Husbandry Division swine herd at Beltsville, Maryland. The results indicated that killed virus vaccines used in this manner had excellent immunizing properties.

Killed virus vaccine is not a source of live virus which could be maintained in the pig for possible transmission to susceptible swine at a later time. This factor would be very important in any hog cholera eradication program. To test the theory that all live hog cholera virus could be eliminated from an enzootic hog cholera area while maintaining a highly...
resistant swine population, a vaccination and regulatory project was de-
volved for Lowndes County, Georgia.

Lowndes County, Georgia, was selected for a three-year killed virus
vaccination study because it is located in an enzootic hog cholera area. A
severe enzootic outbreak of hog cholera had occurred in the county during
the summer of 1960 and some cases were being diagnosed when the vacci-
nation program was begun in 1962. The county also is 50 miles from the
United States Department of Agriculture Hog Cholera Research Station at
Live Oak, Florida, where all challenge work was to be done. The county
is located on the Florida-Georgia line approximately halfway between the
Atlantic Coast and the Alabama-Georgia border. The soil is mostly sand
with several large swampy areas. A portion of the northern part is clay
soil and higher than the surrounding sandy areas. The county line is
formed by rivers on the entire west border, a small part of the south, and
approximately one-third of the east border. Wild swine run free in
Lowndes County and in surrounding counties and may serve as a live virus
reservoir. A sale barn and local packing plant are located in Valdosta, the
county seat. Most of the 50 to 150 feeder pigs and breeding swine from
other counties sold through the sale barn each week, go to farms in
Lowndes County. Very few of these pigs are vaccinated prior to sale and
some are sold by farmers located close to a hog cholera outbreak. Feeder
pigs and breeding stock are also purchased at surrounding county sale
barns and markets by Lowndes County farmers. These are usually non-
vaccinated swine some of which may have been exposed to hog cholera.

MATERIALS AND METHODS

Boynton's tissue vaccine and crystal violet glycerol vaccine were
used in the vaccination program. Lowndes County was divided into seven
areas (Figure 1).

Vaccination consisted of the injection of two five-ml. doses of the as-
signed vaccine 30 days apart. Vaccines were furnished free to the veteri-
narians and the cost of their services was paid from project funds. Non-
vaccinated swine returning to Lowndes County farms from the Lowndes
County market and the three markets in surrounding counties were vac-
cinated with the initial dose of killed virus vaccine and 15 ml. to 30 ml. of
anti-hog cholera serum determined by the weight of the pig. Serum costs
were paid by the swine owner. Nonvaccinated swine from other Georgia
markets were treated with anti-hog cholera serum before leaving the
market and vaccinations were completed on the farm under quarantine.
Killed virus vaccines used in the project were tested for potency by live
virus challenge of vaccinated hogs prior to use in the field. Immunizing
effectiveness of one- and two-dose killed virus vaccine used with anti-hog
cholera serum was tested prior to field use; serum was given with the
first dose of vaccine.

Local practicing veterinarians vaccinated as many pigs as they had
time to vaccinate and other swine were vaccinated by veterinarians of the
Animal Disease Eradication Division and the State of Georgia. Local
Figure 1. Map of Lowndes County showing vaccination areas.

*Boyton's tissue vaccine (BTV) manufactured by Cuttler Laboratories, Berkeley, California, and Haver Lockhart Laboratories, Kansas City, Missouri.
**Experimental crystal violet glycerol vaccine (ECVG) manufactured by National Animal Disease Laboratory, Ames, Iowa.
***Commercial crystal violet glycerol vaccine (CCVG) manufactured by Colorado Serum Company, Denver, Colorado.

personnel, Animal Disease Eradication Division personnel, and State lay inspectors lined up swine for vaccination to further expedite a rapid and complete coverage.

The first dose of vaccine was administered to pigs two weeks of age and older and the second was given 30 days later. Three nonvaccinated pigs were left in each herd of 20 or more pigs when the first dose of
vaccine was given. Two of these pigs were given a single dose of vaccine when the herd was given the second dose of vaccine and one pig was left as a susceptible control to detect the possible exposure of the herd to hog cholera virus. The two single-vaccinated pigs were left to give data on the effectiveness of a single dose of vaccine compared to two doses of vaccine.

A vaccination form was provided to record pertinent information about pigs vaccinated in this program and an ear-notching system was devised for identification of vaccinated pigs. A vaccination certificate was also provided for the vaccinator's and owner's records.

Nonvaccinated swine going through markets to Lowndes County farms presented an eradication control problem of great importance. To prevent introduction of modified live virus from out-of-county sales, nonvaccinated pigs sold to Lowndes County farmers at the markets in the adjacent counties were treated with the first dose of killed virus vaccine (ECVG) and 15 to 30 ml. of anti-hog cholera serum. Pigs sold in private treaty or through other markets received only anti-hog cholera serum prior to leaving that market. Signs were placed in the auction rings of local sale barns requesting buyers to give destination for all swine going to Lowndes County. Barn inspectors and veterinarians were asked to check the destination of swine to be vaccinated. Newly purchased pigs placed on Lowndes County farms were isolated for 30 days following moving.

Daily sales records were kept of pigs delivered to the Valdosta market indicating their classification as stockers or for slaughter and vaccinated or not vaccinated. Sale tickets were checked to determine if pigs were returned to a farm or were purchased for slaughter and a list of buyers who had swine vaccinated at the barn was obtained from the barn veterinarian. The three reports were compared and buyers removing nonvaccinated swine for other than immediate slaughter were reported to State and Federal officials.

Reported swine problems arising during this vaccination program were investigated. Histories were recorded for all suspicious cases. Information obtained included number of pigs on farm, number sick and dead, vaccination status, contact with other herds, visitors to farm, and contacts of operator and hired help with other herds. Also recorded were number of pigs necropsied, samples and specimens taken, tests performed, and possible source of exposure of affected herds investigated. Diagnostic facilities of the State Animal Disease Laboratory at Tifton, Georgia, the USDA Hog Cholera Research Station at Live Oak, Florida, and the USDA National Animal Disease Laboratory at Ames, Iowa, were used for cases requiring laboratory examination. Diagnosis of hog cholera was based on signs, gross lesions, herd history, white cell counts, brain lesions, and pig inoculation tests.

For the pig inoculation test, spleens from suspect pigs were ground with sterile sand in sterile normal saline solution. The suspension was then centrifuged and the supernatant fluid decanted into a sterile vaccine bottle. Two hog cholera-susceptible pigs and one immune pig were placed in an isolation pen and each was given a five-ml. injection of spleen suspension subcutaneously. One of the susceptible pigs was also given 0.5 ml.
of commercial anti-hog cholera serum per pound of live weight simultaneously with the spleen suspension. Pigs were observed daily for changes in feed consumption. If no visible reaction occurred during the 14 days following injection, the immune pig was released and the pig which received spleen suspension only was placed in the challenge barn and injected with one ml. of virulent hog cholera virus. The pig receiving spleen suspension and anti-hog cholera serum was challenged with virulent hog cholera virus 30 days after initial injection. A test was considered negative if the pigs receiving spleen suspension only and spleen suspension with anti-hog cholera serum died from virulent virus challenge with signs and lesions of hog cholera, while the hog cholera-immune pig remained healthy. A test was considered positive for hog cholera if the pig receiving spleen suspension only died during the first two weeks, the pig receiving spleen suspension plus anti-hog cholera serum remained healthy after challenge, and the hog cholera-immune pig remained healthy. If the three pigs became sick, some infectious agent other than hog cholera virus was considered to be present.

If hog cholera was diagnosed, all swine involved were quarantined immediately by State of Georgia personnel and not released until 30 days after the last sick pig recovered or died. Healthy exposed pigs could be moved direct to slaughter if trucks were properly cleaned and disinfected after shipment. No pigs could be added to the herd during quarantine.

Monthly reports of hog cholera outbreaks in Georgia were obtained from the State Animal Disease Laboratory, Tifton, Georgia. These reports were used in evaluating the effectiveness of the vaccinations and market controls. Such reports also indicated sources of infection. Swine sold into the country from areas of current outbreaks were closely watched for signs of hog cholera.

Surveys were made by personnel of the State of Georgia and the Animal Disease Eradication Division to determine the vaccination coverage of Lowndes County, to contact farmers who had not joined the program, and to provide an estimate of the current and projected swine population.

Double-vaccinated swine (DVX), single-vaccinated controls (SVX), and nonvaccinated controls (NVX) were purchased at markets or from farmers. These swine were taken to the Hog Cholera Research Station at Live Oak, Florida, for challenge. One hog cholera-susceptible pig was included with each challenge group to check the virulence of the virus. Temperatures of swine were taken on the day of purchase, morning and evening of the day of challenge, and each morning for seven days. Challenge consisted of injecting swine subcutaneously with one ml. of USDA Hog Cholera Research Station virulent virus serial number 1c. Its LD$_{50}$ titer was $10^{-5}$ per five ml. at the last completed titration performed in April, 1963.

Appetites of challenged pigs were observed daily and recorded as good to off-feed as follows:

<table>
<thead>
<tr>
<th>Appetite</th>
<th>Good</th>
<th>Fair</th>
<th>Slight</th>
<th>Off-Feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Points</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Abnormalities such as lameness which could affect appetites were noted. Swine which maintained good appetites were released the 11th day after challenge. Swine with depressed appetites were held until recorded as having good appetites for three consecutive days before release. After release, swine were moved to a barn for immune swine and held for at least two weeks. A susceptible pig was placed in the released group for one week for detection of virus shedding. Surviving healthy swine were sold for slaughter. All pigs which died after challenge were examined for gross lesions of hog cholera. Swine which did not have a loss of appetite were considered to be 100 percent resistant to challenge. For each point given, two percent was deducted from 100 percent. Those pigs accumulating zero through 10 points (100 percent through 80 percent protection) were classed as having none to a slight reaction. From 11 through 49 points (78 percent through two percent protection) was considered a severe reaction, and those pigs getting 50 or more points or dying were considered to have zero percent protection.

RESULTS

Lowndes County pigs received their first dose of vaccine during the first month of the program. The average vaccination herd contained approximately 20 pigs. By June 30, 1963, 96,107 doses of vaccine had been used in 5,007 vaccination groups or herds (Tables Ia and Ib). Of those swine vaccinated, 53,027 received the first vaccination, Table Ia, and 43,080 or 81.2 percentage were vaccinated twice, Table Ib. The percentage of combined first and second vaccinations according to the type of vaccine used was BTV 19.9 percent, ECVG 42.2 percent, and CCVG 37.9 percent.

Vaccination rates ranged from a low of 1,330 first and 885 second vaccinations for June, 1962, to a high of 4,764 first and 2,542 second vaccinations for March, 1963 (Figure 2). Vaccinations for 1963 remained higher than for the same period of 1962.

Market reports kept by all co-operating markets revealed that 67.2 percent of Lowndes County swine were vaccinated prior to sales (Table II). The total swine marketed and reported represented 53.2 percent of the 28,035 first vaccinates treated during the same period of July 1, 1962, through June 30, 1963. Using 20,000 swine as an estimate of the swine population of Lowndes County, 74.6 percent of the population was accounted for through the market reports.

During the period July 1, 1962, through June 30, 1963, 3,933 swine originating from out of the county were delivered to Lowndes County markets for sale (Table III). Of these swine, 91.4 percent had not been vaccinated before sale delivery. The nonvaccinated group included 1,356 feeder swine. Approximately 55 percent of all feeder and breeder swine sold in Lowndes County are bought by Lowndes County buyers and remain in this county. Nineteen buyers removed 169 nonvaccinated swine for other than immediate slaughter (Table IV). A total of 8,493 nonvaccinated swine were sold at Lowndes County markets; therefore, only two percent were removed from the markets without vaccination.
TABLE Ia
Number of Swine Given First Vaccination for Period
January 29, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>BTV</th>
<th>ECVG</th>
<th>CCVG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Line a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds Pigs</td>
<td>118</td>
<td>4476</td>
<td>324</td>
<td>8334</td>
</tr>
<tr>
<td>Herds Pigs</td>
<td>296</td>
<td>7124</td>
<td>738</td>
<td>19934</td>
</tr>
<tr>
<td>Grand Total</td>
<td>427</td>
<td>9996</td>
<td>983</td>
<td>20757</td>
</tr>
</tbody>
</table>

| Mar 1, 1962 Through June 30, 1963 |          |            |            |             |
| Herds Pigs      | 309        | 5520       | 659        | 12423       |
| Herds Pigs      | 696        | 11422      | 1664       | 29365       |
| Grand Total     | 385        | 3728       | 385b       | 3728b       |

aTotal swine and herds vaccinated on Lowndes County, Georgia farms from January 29, 1962 through February 28, 1962.

bTotal first vaccinations plus serum and killed virus vaccination equals 2787 herds and 53,027 swine.

TABLE Ib
Number of Swine Given Second Vaccination for Period
March 1, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>BTV</th>
<th>ECVG</th>
<th>CCVG</th>
<th>Mixed Vaccines a Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Line b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds Pigs</td>
<td>124</td>
<td>3687</td>
<td>416</td>
<td>8985</td>
</tr>
<tr>
<td>Herds Pigs</td>
<td>301</td>
<td>7426</td>
<td>--</td>
<td>841</td>
</tr>
<tr>
<td>Grand Total</td>
<td>391</td>
<td>8225</td>
<td>881</td>
<td>17868</td>
</tr>
</tbody>
</table>

aVaccine for second vaccination of different type than that used for first vaccination.
bTotal swine and herds vaccinated on Lowndes County, Georgia farms from March 1, 1962 through March 31, 1962.

TABLE II
Vaccinations of Lowndes County Swine Sold Through Livestock Markets
from July 1, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Swine</th>
<th>Slaughter</th>
<th>Feeder</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>7,857</td>
<td>74.6</td>
<td>2,160</td>
</tr>
<tr>
<td>Not Vaccinated</td>
<td>2,680</td>
<td>25.4</td>
<td>2,217</td>
</tr>
<tr>
<td>Total</td>
<td>10,537</td>
<td>100.0</td>
<td>4,377</td>
</tr>
</tbody>
</table>
Figure 2. Swine vaccinations by month after completion of initial coverage.
TABLE III
Sales of Out-of-County Swine in Lowndes County
July 1, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Swine</th>
<th>Slaughter</th>
<th></th>
<th>Feeder</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Vaccinated prior to sale</td>
<td>266</td>
<td>10.6</td>
<td>71</td>
<td>5.0</td>
<td>337</td>
<td>8.6</td>
</tr>
<tr>
<td>Not vaccinated prior to sale</td>
<td>2240</td>
<td>89.4</td>
<td>1356</td>
<td>95.0</td>
<td>3596</td>
<td>91.4</td>
</tr>
<tr>
<td>Grand Total</td>
<td>2506</td>
<td>100.0</td>
<td>1427</td>
<td>100.0</td>
<td>3933</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TABLE IV
Nonvaccinated Swine Removed from Lowndes County Markets for Other than Immediate Slaughter,
July 1, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Violators</th>
<th>Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Removed by Lowndes County Farmers</td>
<td>Number</td>
</tr>
<tr>
<td>15</td>
<td>78.9</td>
</tr>
<tr>
<td>Removed by Out-of-County Farmers</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
</tr>
</tbody>
</table>

TABLE V
Laboratory-Diagnosed Hog Cholera Outbreaks in Lowndes County, Georgia, and Adjoining Georgia and Florida Counties

<table>
<thead>
<tr>
<th>Number of Outbreaks</th>
<th>Lowndes County</th>
<th>Adjoining Counties</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 1, 1962 through December 31, 1962</td>
<td>1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>January 1, 1963 through June 30, 1963</td>
<td>1&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup>Georgia outbreaks diagnosed by the State Animal Disease Laboratory, Tifton, Georgia.
<sup>b</sup>Georgia outbreaks diagnosed by the State Animal Disease Laboratory, Tifton, Georgia and confirmed with pig inoculation test by the USDA Hog Cholera Research Station, Live Oak, Florida.
<sup>c</sup>Negative by pig inoculation tests at the USDA Hog Cholera Research Station, Live Oak, Florida and the National Animal Disease Laboratory, Ames, Iowa.
<sup>d</sup>Florida outbreaks diagnosed by USDA Hog Cholera Research Station, Live Oak, Florida with pig inoculation tests.
<sup>e</sup>Florida outbreaks diagnosed by the state Diagnostic Laboratory, Kissimmee, Florida.
Since the start of vaccinations in Lowndes County, the State Animal Disease Laboratory, Tifton, Georgia, has reported 312 confirmed cases of hog cholera in Georgia. Twenty-four cases have occurred in the counties adjoining Lowndes including 20 cases in Georgia counties and four cases in adjoining Florida counties (Table V). Seventy-seven investigations of sick swine in Lowndes County were made. Signs and lesions found during nine of those investigations indicated hog cholera might be involved. Specimens and sick swine were submitted to the State Animal Disease Laboratory, Tifton, Georgia. Portions of specimens from nine suspected outbreaks were also tested by pig inoculations at the USDA Hog Cholera Research Station, Live Oak, Florida. Seven of the nine suspicious cases were negative for hog cholera with tests done at both laboratories. Two cases were diagnosed positive in Lowndes County by the State Animal Disease Laboratory, Tifton, Georgia. One case was confirmed as positive and one case was proved negative by pig inoculation tests at the USDA Hog Cholera Research Station and National Animal Disease Laboratory, Ames, Iowa (Table V).

Due to the small number of pigs vaccinated in the fall and early winter of 1962, a survey of Lowndes County farmers was made by Animal Disease Eradication Division personnel during the month of February, 1963 (Tables VIa and VIb). Of those farmers contacted, 56.3 percent owned swine. A total of 18,478 swine, including 600 sows due to farrow in 30 to 90 days were owned by these farmers. Nonvaccinated swine...
represented 43.7 percent of the total. At the time of the survey, less than 70 percent of swine sold through the Lowndes County markets were vaccinated prior to marketing.

Increased inspection and regulation of Lowndes County garbage feeders was initiated in February, 1963. Thirty-one garbage feeders are operating at present in Lowndes County. Those who did not comply with cooking and sanitation requirements were forced to stop feeding garbage. Feeders still operating are inspected twice monthly, or more often if conditions warrant.

From May 29, 1962, to June 30, 1963, 1,076 killed virus-vaccinated swine were exposed to virulent virus. Average percentage of protection for the entire group was 79.8 percent. Double-vaccinated swine, vaccinated as weanlings, averaged 84.9 percent protection as compared with 70.7 percent protection for single-vaccinated weanlings (Table VII). Double-vaccinated suckling pigs averaged 76.4 percent protection while single-vaccinated suckling pigs averaged 60.2 percent protection (Table VIII). Double-vaccinated weanlings receiving anti-hog cholera serum with the first dose of vaccine had an average of 71.2 percent protection (Table IX). All double-vaccinated swine challenged average 82.1 percent protection while all single-vaccinated swine challenged averaged 69.6 percent protection (Table X).

Results of challenge by the month of double-vaccinated and single-vaccinated swine are shown in Figure 3. Sixty-nine nonvaccinated swine

*Includes one challenge group completed in May 1962.
<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Adequately Protected (Negative or Slight Reactions 0 - 10 Points 100% - 80% Protection)</th>
<th>Survival (Survived and Released Apparently Normal 0 - 49 Points 100% - 2% Protection)</th>
<th>Died</th>
<th>Adequately Protected (Negative or Slight Reactions 0 - 10 Points 100% - 80% Protection)</th>
<th>Survival (Survived and Released Apparently Normal 0 - 49 Points 100% - 2% Protection)</th>
<th>Died</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boynton's Tissue</td>
<td>Number of Pigs: 63/85</td>
<td>Percent of Pigs: 74.1</td>
<td>Number of Pigs: 76</td>
<td>Percent of Pigs: 89.4</td>
<td>Number of Pigs: 9/85</td>
<td>Percent of Pigs: 10.6</td>
<td>Number of Pigs: 10.6</td>
</tr>
<tr>
<td>Experimental CVG</td>
<td>Number of Pigs: 287/365</td>
<td>Percent of Pigs: 78.6</td>
<td>Number of Pigs: 337</td>
<td>Percent of Pigs: 92.3</td>
<td>Number of Pigs: 28</td>
<td>Percent of Pigs: 7.7</td>
<td>Number of Pigs: 7.7</td>
</tr>
<tr>
<td>Commercial CVG</td>
<td>Number of Pigs: 122/137</td>
<td>Percent of Pigs: 89.1</td>
<td>Number of Pigs: 132</td>
<td>Percent of Pigs: 96.4</td>
<td>Number of Pigs: 5</td>
<td>Percent of Pigs: 3.6</td>
<td>Number of Pigs: 3.6</td>
</tr>
<tr>
<td>Total</td>
<td>Number of Pigs: 472/587</td>
<td>Percent of Pigs: 80.4</td>
<td>Number of Pigs: 545</td>
<td>Percent of Pigs: 92.8</td>
<td>Number of Pigs: 42</td>
<td>Percent of Pigs: 7.2</td>
<td>Number of Pigs: 7.2</td>
</tr>
</tbody>
</table>

\( ^a \) Numerator - number of pigs with negative or slight reactions after challenge  
Denominator - number of pigs challenged  

\( ^b \) Numerator - number of pigs which survived after challenge and were released apparently normal  
Denominator - number of pigs challenged  

\( ^c \) Numerator - number of pigs which died after challenge  
Denominator - number of pigs challenged
TABLE VIII
Challenge Results of Swine Vaccinated as Sucklings from May 29, 1963 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Double Vaccinates</th>
<th>Survival</th>
<th>Adequately Protected (Negative or Slight Reactions 0 - 10 Points 100% - 80% Protection)</th>
<th>Died</th>
<th>Percent Protection</th>
<th>Single Vaccinates</th>
<th>Survival</th>
<th>Adequately Protected (Negative or Slight Reactions 0 - 10 Points 100% - 80% Protection)</th>
<th>Died</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boynton's Tissue</td>
<td>29/47</td>
<td>61.7</td>
<td>40/47</td>
<td>85.1</td>
<td>7/47</td>
<td>14.9</td>
<td>71.7</td>
<td>0/6</td>
<td>0.0</td>
<td>140</td>
</tr>
<tr>
<td>Experimental CVG</td>
<td>111/172</td>
<td>64.5</td>
<td>140/172</td>
<td>81.4</td>
<td>32/172</td>
<td>18.6</td>
<td>71.5</td>
<td>7/11</td>
<td>63.6</td>
<td>9/11</td>
</tr>
<tr>
<td>Commercial CVG</td>
<td>55/62</td>
<td>88.7</td>
<td>61/62</td>
<td>98.4</td>
<td>1/62</td>
<td>1.6</td>
<td>93.6</td>
<td>1/1</td>
<td>100.0</td>
<td>1/1</td>
</tr>
<tr>
<td>Total</td>
<td>195/281</td>
<td>69.4</td>
<td>241/281</td>
<td>85.8</td>
<td>40/281</td>
<td>14.2</td>
<td>76.4</td>
<td>8/18</td>
<td>44.4</td>
<td>14/18</td>
</tr>
</tbody>
</table>

aNumerator - number of pigs with negative or slight reactions after challenge
Denominator - number of pigs challenged
bNumerator - number of pigs which survived after challenge and were released apparently normal
Denominator - number of pigs challenged
cNumerator - number of pigs which died after challenge
Denominator - number of pigs challenged
TABLE IX
Challenge Results of Pigs Double Vaccinated as Weanlings and Older with Experimental CVG Vaccine Simultaneously with Serum at First Vaccination from May 29, 1962 through June 30, 1963

<table>
<thead>
<tr>
<th>Type of Vaccine Used at Second Vaccination</th>
<th>Number of Pigs(^a)</th>
<th>Percent of Pigs</th>
<th>Number of Pigs(^b)</th>
<th>Percent of Pigs</th>
<th>Number of Pigs(^c)</th>
<th>Percent of Pigs</th>
<th>Protection (Negative or Slight Reactions 0 - 10 Points 10% - 80% Protection)</th>
<th>Survival (Survived and Released Apparently Normal 0 - 49 Points 100% - 2% Protection) Died</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boynton's Tissue</td>
<td>0/1</td>
<td>0.0</td>
<td>0/1</td>
<td>0.0</td>
<td>1/1</td>
<td>100.0</td>
<td>0.0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Experimental CVG</td>
<td>19/29</td>
<td>65.5</td>
<td>25/29</td>
<td>86.2</td>
<td>4/29</td>
<td>13.8</td>
<td>75.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial CVG</td>
<td>5/11</td>
<td>45.5</td>
<td>9/11</td>
<td>81.8</td>
<td>2/11</td>
<td>18.2</td>
<td>66.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24/41</td>
<td>58.5</td>
<td>34/41</td>
<td>82.9</td>
<td>7/41</td>
<td>17.1</td>
<td>71.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Numerator - number of pigs with negative or slight reactions after challenge
Denominator - number of pigs challenged

\(^b\)Numerator - number of pigs which survived after challenge and were released apparently normal
Denominator - number of pigs challenged

\(^c\)Numerator - number of pigs which died after challenge
Denominator - number of pigs challenged
### TABLE X
Comparison of Challenge Results of Double-Vaccinated Swine and Single-Vaccinated Swine

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Double Vaccinates</th>
<th>Single Vaccinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adequately Protected (Negative or Slight Reactions)</td>
<td>Survival (Survived and Released Apparently Normal)</td>
</tr>
<tr>
<td></td>
<td>Number of Pigs</td>
<td>Percent of Pigs</td>
</tr>
<tr>
<td>Boynton's Tissue</td>
<td>92/132</td>
<td>69.7</td>
</tr>
<tr>
<td>Experimental CVG</td>
<td>398/537</td>
<td>74.1</td>
</tr>
<tr>
<td>Commercial CVG</td>
<td>177/199</td>
<td>88.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>667/868</td>
<td>76.8</td>
</tr>
</tbody>
</table>

**Note:**
- Numerator - number of pigs with negative or slight reactions after challenge
- Denominator - number of pigs challenged
- Numerator - number of pigs which survived after challenge and were released apparently normal
- Denominator - number of pigs challenged
- Numerator - number of pigs which died after challenge
- Denominator - number of pigs challenged
TABLE XI
Comparison of Challenge Results of Normal Swine and Those Suffering from Stress Factors or Parasitism at Time of Vaccination

<table>
<thead>
<tr>
<th>Type of Swine</th>
<th>Adequately Protected (Negative or Slight Reactions)</th>
<th>Survival (Survived and Released Apparently Normal)</th>
<th>Died</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pigs</td>
<td>Percent of Pigs</td>
<td>Number of Pigs</td>
<td>Percent of Pigs</td>
</tr>
<tr>
<td>Stressed or Parasitism</td>
<td>47/68</td>
<td>69.1</td>
<td>64/68</td>
<td>94.1</td>
</tr>
<tr>
<td>Normal</td>
<td>747/1008</td>
<td>74.1</td>
<td>889/1008</td>
<td>88.2</td>
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<tr>
<td>Total</td>
<td>794/1076</td>
<td>73.8</td>
<td>953/1076</td>
<td>88.6</td>
</tr>
</tbody>
</table>

\(^a\)Numerator - number of pigs with negative or slight reactions after challenge
Denominator - number of pigs challenged

\(^b\)Numerator - number of pigs which survived after challenge and were released apparently normal
Denominator - number of pigs challenged

\(^c\)Numerator - number of pigs which died after challenge
Denominator - number of pigs challenged
TABLE XII
Comparison of Average Percent Protection of Double Vaccinated Suckling Pigs
According to Type of Vaccine Used

<table>
<thead>
<tr>
<th>Vaccination of Sow</th>
<th>BTV</th>
<th>ECVG</th>
<th>CCVG</th>
<th>Killed Virus Total</th>
<th>MLV#</th>
<th>NVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Pigs</td>
<td>No. of Pigs</td>
<td>Per- cent Protection</td>
<td>No. of Pigs</td>
<td>Per- cent Protection</td>
<td>No. of Pigs</td>
<td>Per- cent Protection</td>
</tr>
<tr>
<td>BTV</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>ECVG</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>32</td>
<td>94</td>
</tr>
<tr>
<td>CCVG</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>49</td>
<td>27</td>
<td>69.0</td>
<td>87.5</td>
<td>74.6</td>
</tr>
</tbody>
</table>

#All types of modified live virus which were used for vaccination.

were exposed to virulent virus. Sixty-eight died with signs and lesions of hog cholera.

Sixty-eight swine classified as being parasitized or having stress factors, such as poor diet, poor sanitation, and respiratory diseases were exposed to virulent virus. These 68 swine after challenge had an average percentage of protection of 81.4 percent as compared with 79.7 percent for other swine challenged (Table XI).

Suckling pigs were given one and/or two doses of the same killed virus vaccine that the dams were vaccinated with and exposed to hog cholera virus. Also pigs from sows vaccinated with modified live vaccines and nonvaccinated sows were vaccinated with the three killed virus vac- cines and exposed to hog cholera virus. The comparative results are given in Table XII.

DISCUSSION

The principal objective of a large vaccination project is the effective immunization of all members of the population. This is not feasible at the present time because certain individuals cannot be vaccinated for various reasons and others which are vaccinated will not develop immunity. Therefore, some susceptible members are usually present at any given time to serve as the nucleus for a disease outbreak. Protection against exposure must serve as the means of prevention for the remaining susceptible members. The protection against exposure can be accomplished by preventing contact of susceptible members with carriers or sick individuals and by their isolation from other individuals for enough time to prevent passage of the disease agent.

The vaccination rate of Lowndes County swine varied widely since completion of the initial vaccination in April, 1962. The rate of vaccination was very low during the summer and fall of 1962. This was due to apathy on the part of the swine raisers, the need for more line-up and vaccination help, and that the tobacco harvest was not yet com-
suspected outbreak in Lowndes County, and additional line-up and vaccination help maintained a higher vaccination rate for the summer of 1963. The survey by personnel of the Animal Disease Eradication Division in February, 1963, revealed that, of the farmers contacted, 43.7 percent of their pigs had not been vaccinated. Due to the renewed interest among swine owners and use of extra vaccination and line-up help, the number of pigs vaccinated increased rapidly to 85 percent of the swine population.

The period of very low vaccination numbers stressed the importance of an effective control system to prevent introduction of hog cholera virus from out of the county. The effectiveness of the control system was shown in part by allowing only two percent of the nonvaccinated swine sold in Lowndes County markets to go to farms without being vaccinated. Feeder swine make up the bulk of nonvaccinated swine sold in Lowndes County. Many farmers in Lowndes County do not vaccinate until weaning age, which is the time that feeders usually are sold. Rather than bother with vaccination, the pigs frequently are sold nonvaccinated, although double-treated feeders will average one dollar per hundred pounds body weight more than nonvaccinated feeders at a sale. If a very high level of feeder vaccinations prior to marketing could be maintained, the chances of nonvaccinated swine returning to the farm would correspondingly be reduced.

The low incidence of hog cholera in Lowndes County compared to the greater incidence in surrounding counties emphasizes the effectiveness of the Lowndes County eradication program. After each new outbreak, pigs from the affected area are sold at the Lowndes County market. Most of these swine are not vaccinated for hog cholera and some may have been exposed in the outbreak.

Survival of NVX control swine on the farm indicated that these animals were not exposed to virulent hog cholera virus. Since the NVX controls were obtained from farms scattered over Lowndes County, it appears that virulent hog cholera virus in Lowndes County has been virtually eliminated. The deaths of 68 or 69 NVX swine when challenged with virulent virus indicated that these swine were susceptible to hog cholera and would have become infected if exposed naturally. The challenge results also indicate that these pigs were not exposed to a transmissible hog cholera immunizing virus.

Two outbreaks of hog cholera were diagnosed in Lowndes County since vaccination was started, the first on February 17, 1962. The owner had been treating sick swine and burying dead swine on the farm for several weeks. The swine involved were vaccinated for the first time three days prior to the outbreak, indicating infection prior to vaccination. Diagnosis of hog cholera was confirmed by the State Animal Disease Laboratory at Tifton, Georgia, and the USDA Hog Cholera Research Station, Live Oak, Florida.

The second diagnosed case occurred May 29, 1963. Fifty-five nonvaccinated weanlings were involved; they had been nursing BTV-vaccinated sows and were to receive the first dose of BTV the day of the outbreak. These swine were with a group of 140 vaccinated shoats and sows which included two NVX controls. No signs of sickness were exhibited by
the vaccinated swine nor the two NVX controls during this outbreak. Hog cholera was diagnosed by the State Animal Disease Laboratory, Tifton, Georgia and *Salmonella* and *Pasteurella* organisms were also isolated from submitted specimens. Diagnosis of hog cholera was made on the basis of clinical signs reported, gross necropsy lesions, a marginal leukopenia in two pigs and a moderate lymphocytic perivascular cuffing in one brain of five pigs taken to the diagnostic laboratory. Negative pig inoculation tests were obtained at two laboratories, The United States Department of Agriculture Hog Cholera Research Station, Live Oak, Florida and the National Animal Disease Laboratory, Ames, Iowa, when spleen suspensions from the same five pigs were tested in susceptible farm pigs and specific pathogen-free (SPF) pigs respectively. Nine of the 55 sick swine died, but the remainder recovered within two weeks after treatment with anti-hog cholera serum and antibiotics.

Very few overt reactions to vaccinations have occurred, but some problems with lamenesses and injection abscesses have resulted. Most of these cases occurred in parasitized or unthrifty pigs being raised in unsanitary surroundings.

Many swine in Lowndes County were in poor condition and eating a very poor diet at the time of one or both vaccinations. These factors might be expected to lower the immune response to killed virus vaccination. However, the challenge results indicate that neither poor diet nor condition had an adverse effect on immune response.

The three types of killed virus vaccines being used in Lowndes County appeared to stimulate adequate protection against hog cholera in susceptible swine if two doses were given one month apart. Results thus far indicate that, of the three vaccines used, CCVG vaccine was most effective in immunizing suckling and weanling pigs.

Suckling swine are often left with the sow until three to four months of age by Lowndes County swine owners. This may account in part for the results shown by challenge of suckling swine with virulent virus. In some herds suckling may mean only that the pigs are in the same lot with the sows and that the immunity of the sow no longer has any influence on the hog cholera immunity of the pigs. However, challenge results in Table XII show that ECVG-vaccinated pigs nursing MLV-vaccinated sows had better protection than ECVG-vaccinated pigs nursing nonvaccinated sows. Passive immunity to hog cholera in the pig has been shown to be detrimental to the production of an adequate permanent immunity when vaccinating with killed virus vaccines. However, the challenge results of suckling swine vaccinated with CCVG vaccine while nursing CCVG-vaccinated sows indicate that double vaccination may overcome this limitation and provide adequate protection against hog cholera without using some form of live virus which could be passed to other susceptible swine.

The consistently greater degree of protection of double-vaccinated pigs when challenged, compared to single-vaccinated pigs which received the same type of vaccine, indicated the effectiveness of double vaccination.
The incomplete results compiled from the Lowndes County project using killed virus vaccines indicated that two five-ml. doses of killed virus vaccine given one month apart were effective in the control of hog cholera. Challenge data of pigs vaccinated with killed virus vaccine indicated that double vaccination of suckling pigs produced greater protection against hog cholera than single vaccination. The same data also indicate that double vaccination of weanlings was significantly more effective for production of immunity than a single vaccination. No deaths or sickness caused by hog cholera virus were reported in NVX control swine, indicating relative freedom from virulent virus in Lowndes County. Susceptibility of NVX swine indicated that these pigs were not exposed to transmissible immunizing hog cholera virus. A combination of a highly resistant swine population created by use of killed virus vaccine and strict market controls has proved to be of value for eradication of hog cholera from Lowndes County.

REFERENCES

A FIVE-YEAR STUDY OF FARM SWINE HERDS VACCINATED WITH CRYSTAL VIOLET GLYCEROL HOG CHOLERA VACCINE*

J. P. Torrey, B.S., D.V.M., M.S.
Ames, Iowa

This report concludes a five-year study (1956-1960) of farm swine herds vaccinated against hog cholera with crystal violet glycerol (CVG) vaccine; two preliminary reports were made.¹,³

The objectives of the study were to determine: (1) the ability of pigs vaccinated at the farm to withstand exposure to virulent hog cholera (HC) virus at one, three, and six months after vaccination, (2) the factors responsible for the inability of vaccinated pigs to withstand exposure to virulent HC virus, (3) the efficacy of a yearly vaccination with CVG vaccine in herds on the same farms for the prevention of naturally occurring hog cholera, and, (4) the efficacy of double vaccination of pigs that did not develop satisfactory immunity against exposure to virulent HC virus after a single vaccination.

MATERIALS AND METHODS

The stock HC virus was a strain that had been maintained for many years at the Federal Hog Cholera Research Station, Ames, Iowa. Virulent HC virus for challenge and vaccine production was prepared each year by passing the stock virus through pigs. Each lot had an approximate titer of 500,000 MLD per ml. and was free from variant characteristics.²

The preparation of CVG vaccine used in this study has been described.¹ All vaccines were tested in hog cholera-susceptible pigs and approved before they were used on farm herds. Approval depended upon one-half of the recommended dose of vaccine, or 2.5 ml., protecting pigs against two ml. of challenge hog cholera virus 21 days after vaccination.

Vaccination of farm herds was done at the request of the owner without solicitation or selection as to location. These farms were located in six counties in Iowa. Vaccination was carried out on 93 farms the first year, but the number declined to 60 farms by the end of the fifth year. This reduction in numbers was due to owners either moving from the community or quitting swine raising. Although there was a decrease in the number of farms, the number of hogs vaccinated did not decrease. Spring

*From National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa. The author gratefully acknowledges the assistance of Drs. G. H. Barney and M. Marmesh, formerly of this division.
and fall litters on a farm were considered as separate herds. Most of the hogs on these farms were either hybrids or crossbreds.

A total of 67,058 pigs was vaccinated in the five years. The number of pigs vaccinated in 1956, Table I, is not the same as previously reported, because it had not been decided when the records for a year should begin and end. Some pigs vaccinated in 1955, but tested in 1956, were included in the first report. Later, swine were divided on an annual basis to simplify the method of identifying the individual herds over a five-year period.

Most pigs were vaccinated about two weeks after weaning at an average age of 9.2 weeks. Each pig was given five ml. of CVG vaccine subcutaneously near the point of the elbow. Gilts and sows retained for breeding were revaccinated either before breeding or when their litters were vaccinated.

When sick or dead pigs were reported in a farm herd after vaccination, post-mortem examinations were made and either splenic suspensions or blood was injected into susceptible pigs to determine if HC virus was present.

During the first three years of the study, two pigs were purchased from each herd one, three, and six months after vaccination for exposure to HC virus. The purchase of six-month vaccinated pigs was discontinued during the third year for lack of funds. Each pair of pigs was delivered to the Federal Hog Cholera Research Station, placed in a small isolation pen within a barn and injected subcutaneously with two ml. of virulent HC virus. Daily observations were made and the condition of each pig was recorded by a point system. Pigs that did not show a reaction were held under observation for two weeks; others were observed until they either died or recovered. A post-mortem examination was conducted on pigs that died, and the cut surface of portions of the heart, lungs, spleen, kidney and liver from each pig was smeared on Proteose Peptone Number Three agar plates (Difco). The plates were examined after 48 hours' incubation at 37 C. for the presence of significant bacterial growth. Subcultures of organisms representing selected colonies were studied and placed in their generic classification but no attempt was made to determine the species. A total of 2,931 pigs or 4.37 percent of the vaccinated pigs was exposed to virulent HC virus. There were 1,236 pigs exposed at one month, 1,229 at three months, and 466 at six months after vaccination.

The point system for recording and evaluating the reaction to challenge was as follows: no points if the pig remained normal, one point if the pig did not eat a normal daily ration and was somewhat slow in eating, two points if the pig ate only a small amount of feed and soon went back to the nest, and three points if the pig refused to eat and stayed in the nest. This point system was then converted to a descriptive expression of reaction, percentage of reaction range, and protection as follows:
Two approaches were made in order to evaluate the efficacy of CVG vaccine; (1) the range of the percentage of reaction was divided into four categories as follows: 80-100 = good protection, 60-79 = fair protection, 1-59 = poor protection, and 0 = no protection, and (2) the percentage of protection was determined for each group of one, three, and six-month vaccinated pigs by adding together the minimum percentage of the range of reaction for each animal (including those that died) and dividing this figure by the total number of animals in that group for each year and finally for five years.

A review of the five-year records showed that all of the test pigs from some farm herds had severe reactions or died each year after exposure to virulent HC virus. In 1960, four of these herds were selected for further study. Bacteriological examinations of the test pigs that died during the five years revealed Pasteurella spp. in each of the four herds. Sanitation and farm management were above average on two of the farms and there was no history of sick pigs. The conditions on the other two farms were not as good and there was a history of death in young pigs as well as unthriftiness and irregular growth. One of these herds was kept in a cattle feeding lot after the pigs weighed 100 pounds and death continued to occur among the unthrifty pigs.

The pigs of the four selected herds had been vaccinated when eight to nine weeks old and two pigs from each herd had severe reactions or died when exposed to HC virus one month after vaccination. When the pigs were 15 to 23 weeks old, 10 more pigs were selected from each herd for exposure to HC virus and the remainder of each herd was given a second injection of CVG vaccine. One month after the second injection of CVG vaccine, 10 pigs were selected from each herd and exposed to HC virus.

RESULTS

The reactions of pigs vaccinated with a single dose of CVG vaccine to challenge with virulent HC virus one, three, and six months after vaccination demonstrated that there was some variation in the level of immunity from year to year. The percentage of pigs in the normal, slight, and dead reactor groups was usually greater in the one-month vaccinated pigs than in the three- and six-month vaccinated pigs. Conversely, the percentage...
### TABLE I

Reaction of CVG-Vaccinated Pigs Exposed to Hog Cholera Virus 1, 3, and 6 Months After Vaccination

<table>
<thead>
<tr>
<th>Year</th>
<th>No. Vac.</th>
<th>No. Tested</th>
<th>Months after Vac.</th>
<th>Normal No. Pigs</th>
<th>Normal %</th>
<th>Slight No. Pigs</th>
<th>Slight %</th>
<th>Moderate No. Pigs</th>
<th>Moderate %</th>
<th>Severe No. Pigs</th>
<th>Severe %</th>
<th>Survived No. Pigs</th>
<th>Survived %</th>
<th>Died No. Pigs</th>
<th>Died %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>11,911</td>
<td>250</td>
<td>1</td>
<td>136</td>
<td>54.40</td>
<td>50</td>
<td>20.00</td>
<td>21</td>
<td>8.40</td>
<td>13</td>
<td>5.20</td>
<td>220</td>
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<td></td>
<td>3</td>
<td>114</td>
<td>46.53</td>
<td>46</td>
<td>18.77</td>
<td>31</td>
<td>12.65</td>
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<td>9.38</td>
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<td>87.34</td>
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<td>12.65</td>
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<td></td>
<td>6</td>
<td>97</td>
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<td>14.22</td>
<td>36</td>
<td>15.51</td>
<td>33</td>
<td>14.22</td>
<td>189</td>
<td>85.77</td>
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<td>14.22</td>
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<tr>
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<td>107</td>
<td>39.92</td>
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<td>23.50</td>
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<td>17.16</td>
<td>31</td>
<td>11.56</td>
<td>247</td>
<td>92.17</td>
<td>21</td>
<td>7.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>50</td>
<td>19.08</td>
<td>77</td>
<td>29.38</td>
<td>53</td>
<td>20.22</td>
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<td>244</td>
<td>93.12</td>
<td>18</td>
<td>6.87</td>
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<td></td>
<td>6</td>
<td>39</td>
<td>18.30</td>
<td>33</td>
<td>15.49</td>
<td>62</td>
<td>29.10</td>
<td>70</td>
<td>32.86</td>
<td>204</td>
<td>95.77</td>
<td>9</td>
<td>4.22</td>
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<tr>
<td>1958</td>
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<td>239</td>
<td>1</td>
<td>23</td>
<td>9.62</td>
<td>62</td>
<td>25.94</td>
<td>58</td>
<td>24.26</td>
<td>45</td>
<td>18.82</td>
<td>188</td>
<td>78.66</td>
<td>51</td>
<td>21.33</td>
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<td>3</td>
<td>25</td>
<td>10.33</td>
<td>38</td>
<td>15.70</td>
<td>60</td>
<td>24.79</td>
<td>74</td>
<td>30.57</td>
<td>197</td>
<td>81.41</td>
<td>45</td>
<td>18.59</td>
</tr>
<tr>
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<td>25.00</td>
<td>11</td>
<td>55.00</td>
<td>3</td>
<td>15.00</td>
<td>19</td>
<td>95.00</td>
<td>1</td>
<td>5.00</td>
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<tr>
<td>1959</td>
<td>14,608</td>
<td>236</td>
<td>1</td>
<td>59</td>
<td>25.00</td>
<td>81</td>
<td>34.32</td>
<td>40</td>
<td>16.94</td>
<td>25</td>
<td>10.59</td>
<td>205</td>
<td>86.86</td>
<td>31</td>
<td>13.13</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>3</td>
<td>55</td>
<td>23.10</td>
<td>70</td>
<td>29.41</td>
<td>48</td>
<td>20.16</td>
<td>39</td>
<td>16.38</td>
<td>202</td>
<td>89.07</td>
<td>26</td>
<td>10.92</td>
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<tr>
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<td>243</td>
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<td>102</td>
<td>41.97</td>
<td>61</td>
<td>25.10</td>
<td>27</td>
<td>11.11</td>
<td>17</td>
<td>6.99</td>
<td>207</td>
<td>85.18</td>
<td>36</td>
<td>14.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>80</td>
<td>33.05</td>
<td>56</td>
<td>23.14</td>
<td>49</td>
<td>20.24</td>
<td>27</td>
<td>11.15</td>
<td>212</td>
<td>87.60</td>
<td>30</td>
<td>12.39</td>
</tr>
<tr>
<td>Five</td>
<td>1236</td>
<td>1</td>
<td>427</td>
<td>34.54</td>
<td>317</td>
<td>25.64</td>
<td>192</td>
<td>15.53</td>
<td>131</td>
<td>10.59</td>
<td>1067</td>
<td>86.33</td>
<td>169</td>
<td>13.67</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>67,058</td>
<td>1229</td>
<td>3</td>
<td>324</td>
<td>26.36</td>
<td>287</td>
<td>23.35</td>
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<td>18.47</td>
<td>1079</td>
<td>87.80</td>
<td>150</td>
<td>12.20</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>6</td>
<td>136</td>
<td>29.18</td>
<td>72</td>
<td>15.45</td>
<td>109</td>
<td>23.39</td>
<td>106</td>
<td>22.74</td>
<td>423</td>
<td>90.78</td>
<td>43</td>
<td>9.22</td>
<td></td>
</tr>
</tbody>
</table>
of pigs in the moderate and severe reactor groups was usually greater in the three- and six-month vaccinated pigs than the one-month vaccinated.

There was, with the exception of 1958, no significant difference between the percentage that survived each year. Of 1,236 pigs exposed to HC virus one month after vaccination, 1,067 (86.33 percent) lived and 169 (13.67 percent) died. Of those that lived the percentage in each reaction classification was: 34.54 percent normal; 25.64 percent slight; 15.5 percent moderate, and 10.51 percent severe. Of 1,229 pigs exposed to HC virus three months after vaccination, 1,079 (87.80 percent) lived and 150 (12.20 percent) died. Of those that lived, the percentage in each reaction classification was: 26.36 percent normal, 23.35 percent slight, 19.60 percent moderate and 18.47 percent severe. Of 466 pigs exposed to HC virus six months after vaccination 423 (90.78 percent) lived and 43 (9.22 percent) died. Of those that lived, the percentage in each reaction classification was: 29.18 percent normal, 15.45 percent slight, 23.39 percent moderate and 22.74 percent severe. (Table I).

The marked increase in the percentage of one- and three-month vaccines that had either severe reactions or died in 1958 was attributed to an infectious bloody diarrhea that originated in pigs obtained from two different farms on two occasions. These pigs, although normal appearing when received, developed a diarrhea after injection with virulent hog cholera virus. This infection subsequently appeared in other pigs in the barn and all affected pigs either died or had severe reactions. Bacteriological examination of the organs of dead pigs and the fecal material from live affected pigs did not reveal a cause for the diarrhea. Inspection of the two farms from which the first pigs with diarrhea originated did not reveal either the presence or history of diarrhea.

The reactions of the pigs tested one, three, and six months after vaccination and expressed as percentage of reaction range of protection further illustrated that there was some variation in the level of immunity. Of a total of 1,236 pigs exposed to HC virus one month after vaccination, the number of pigs in each percentage category was as follows: 100 percent = 427; 80-99 percent = 317; 60-79 percent = 192; 40-59 percent = 77; 20-39 percent = 31; 10-19 percent = nine; five to nine percent = nine; and one to four percent = five. Of 1,229 pigs exposed three months after vaccination, the number of pigs in each percentage category was as follows: 100 percent = 324; 80-99 percent = 287; 60-79 percent = 241; 40-59 percent = 133; 20-39 percent = 48; 10-19 percent = 16; five to nine percent = 10 and one to four percent = 17. Of 466 pigs exposed six months after vaccination the number of pigs in each percentage category was as follows: 100 percent = 136; 80-99 percent = 72; 60-79 percent = 109; 40-59 percent = 65; 20-39 percent = 21; 10-19 percent = seven; five to nine percent = six; and one to four percent = seven (Table II).

Evaluation of the efficacy of CVG vaccine based on the range of percentage of reaction was as follows: of 1,236 pigs tested one month after vaccination 744 (60.18 percent) had good protection, 192 (15.53 percent) had fair protection, 131 (10.51 percent) had poor protection, and 169 (13.67 percent) had no protection. Of 1,229 pigs tested three months after
TABLE II

Percent Range of Reaction of CVG Vaccinated Pigs Exposed to Hog Cholera Virus 1, 3, 6 Months After Vaccination

<table>
<thead>
<tr>
<th>Year</th>
<th>Months after Vac</th>
<th>100%</th>
<th>80-99%</th>
<th>60-79%</th>
<th>40-59%</th>
<th>20-39%</th>
<th>10-19%</th>
<th>5-9%</th>
<th>1-4%</th>
<th>% Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>1</td>
<td>250</td>
<td>136</td>
<td>54.40</td>
<td>50</td>
<td>20.00</td>
<td>21</td>
<td>8.40</td>
<td>4</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>245</td>
<td>114</td>
<td>46.53</td>
<td>46</td>
<td>18.77</td>
<td>31</td>
<td>12.65</td>
<td>12</td>
<td>4.89</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>232</td>
<td>97</td>
<td>41.81</td>
<td>33</td>
<td>14.22</td>
<td>36</td>
<td>15.51</td>
<td>20</td>
<td>8.62</td>
</tr>
<tr>
<td>1957</td>
<td>1</td>
<td>268</td>
<td>107</td>
<td>39.92</td>
<td>63</td>
<td>23.50</td>
<td>46</td>
<td>17.16</td>
<td>20</td>
<td>7.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>262</td>
<td>50</td>
<td>19.08</td>
<td>77</td>
<td>29.38</td>
<td>53</td>
<td>20.22</td>
<td>41</td>
<td>15.64</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>213</td>
<td>39</td>
<td>18.30</td>
<td>33</td>
<td>15.49</td>
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<td>44</td>
<td>20.65</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>242</td>
<td>25</td>
<td>10.33</td>
<td>38</td>
<td>15.70</td>
<td>60</td>
<td>24.79</td>
<td>40</td>
<td>16.52</td>
</tr>
<tr>
<td></td>
<td>6</td>
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<td>5</td>
<td>25.00</td>
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<td>5.0</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td>1959</td>
<td>1</td>
<td>236</td>
<td>59</td>
<td>25.00</td>
<td>81</td>
<td>34.32</td>
<td>40</td>
<td>16.94</td>
<td>17</td>
<td>7.20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>238</td>
<td>55</td>
<td>23.10</td>
<td>70</td>
<td>29.41</td>
<td>48</td>
<td>20.16</td>
<td>27</td>
<td>11.34</td>
</tr>
<tr>
<td>1960</td>
<td>1</td>
<td>243</td>
<td>102</td>
<td>41.97</td>
<td>61</td>
<td>25.10</td>
<td>27</td>
<td>11.11</td>
<td>6</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>242</td>
<td>80</td>
<td>33.05</td>
<td>56</td>
<td>23.14</td>
<td>49</td>
<td>20.24</td>
<td>13</td>
<td>5.37</td>
</tr>
<tr>
<td>Five</td>
<td>1</td>
<td>1236</td>
<td>427</td>
<td>34.54</td>
<td>317</td>
<td>25.64</td>
<td>192</td>
<td>15.53</td>
<td>77</td>
<td>6.22</td>
</tr>
<tr>
<td>Year</td>
<td>3</td>
<td>1229</td>
<td>324</td>
<td>26.36</td>
<td>287</td>
<td>23.35</td>
<td>241</td>
<td>19.60</td>
<td>133</td>
<td>10.82</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>466</td>
<td>136</td>
<td>29.18</td>
<td>72</td>
<td>15.45</td>
<td>109</td>
<td>23.39</td>
<td>65</td>
<td>13.94</td>
</tr>
</tbody>
</table>
vaccination, 611 (49.71 percent) had good protection, 241 (18.24 percent) had fair protection, 244 (18.22 percent) had poor protection, and 150 (12.20 percent) had no protection. Of 466 pigs tested six months after vaccination, 208 (44.63 percent) had good protection, 109 (23.39 percent) had fair protection, 106 (22.74 percent) had poor protection, and 43 (9.22 percent) had no protection (Table III).

**TABLE III**

Evaluation of Protection to Hog Cholera Infection Based on the Percentage Range of Reaction

<table>
<thead>
<tr>
<th>Months after Vac.</th>
<th>Total No. Pigs</th>
<th>Good 89-100%</th>
<th>Fair 60-79%</th>
<th>Poor 1-59%</th>
<th>No 0%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Pigs</td>
<td>Per-cent</td>
<td>No. Pigs</td>
<td>Per-cent</td>
<td>No. Pigs</td>
</tr>
<tr>
<td>1955-60</td>
<td></td>
<td></td>
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<td></td>
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<td>1236</td>
<td>744</td>
<td>60.18</td>
<td>192</td>
<td>15.53</td>
</tr>
<tr>
<td>3</td>
<td>1229</td>
<td>611</td>
<td>49.71</td>
<td>241</td>
<td>19.60</td>
</tr>
<tr>
<td>6</td>
<td>466</td>
<td>208</td>
<td>44.63</td>
<td>109</td>
<td>23.39</td>
</tr>
</tbody>
</table>

Evaluation of the efficacy of CVG vaccine as percentage of protection for the total number of pigs in each group was as follows: 71.91 percent at one month after vaccination, 67.81 percent at three months after vaccination, and 67.27 percent at six months after vaccination (Table IV).

**TABLE IV**

Evaluation of Protection to Hog Cholera Infection as Percentage Protection

<table>
<thead>
<tr>
<th>Months after Vac.</th>
<th>1956 No. Per-cent</th>
<th>1957 No. Per-cent</th>
<th>1958 No. Per-cent</th>
<th>1959 No. Per-cent</th>
<th>1960 No. Per-cent</th>
<th>5 Yr. Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Pigs</td>
<td>Per-cent</td>
<td>No. Pigs</td>
<td>Per-cent</td>
<td>No. Pigs</td>
<td>Per-cent</td>
</tr>
<tr>
<td>1</td>
<td>250</td>
<td>79.57</td>
<td>268</td>
<td>77.34</td>
<td>232</td>
<td>56.35</td>
</tr>
<tr>
<td>3</td>
<td>245</td>
<td>74.67</td>
<td>262</td>
<td>68.03</td>
<td>242</td>
<td>50.53</td>
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<td>6</td>
<td>232</td>
<td>69.88</td>
<td>213</td>
<td>64.67</td>
<td>20</td>
<td>64.45</td>
</tr>
</tbody>
</table>

Average 727 71.81 743 70.42 501 54.35 474 70.61 485 72.40 2931 69.52

Bacteriological examinations were made on 147 pigs that died after exposure to HC virus in 1958, 1959, and 1960 in an effort to determine what factors might be responsible for their inability to withstand the exposure. Bacterial growth was found in 115 or 77.4 percent of the animals. *Pasteurella spp.* was isolated from 58 pigs or 39.45 percent and *Salmonella cholerasuis* was isolated from 25 or 17.0 percent of the animals examined. Miscellaneous organisms were found in the remainder and included other *Salmonella*, *Streptococcus*, *Pseudomonas*, *Proteus*, and coliform organisms.

Hog cholera did not occur in any CVG-vaccinated farm herd during the five-year study. In 1959 and 1960, hog cholera occurred on farms in the area where herds were vaccinated with CVG and, in at least four instances, cholera occurred on farms adjacent to farms where pigs were vaccinated with CVG. The owner of one farm herd did not vaccinate his
pigs after the program was discontinued and eight-week-old pigs on this farm became infected with hog cholera; of 120 pigs, 45 died. The sows and older pigs, which had been vaccinated with CVG vaccine before the program was discontinued, did not become infected.

### TABLE V

Comparison of Single and Double CVG-Vaccinated Pigs Exposed to Hog Cholera Virus

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>1st Vac.</th>
<th>2nd Vac.</th>
<th>1st Exposure</th>
<th>2nd Exposure</th>
<th>Reactions</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 9wks.</td>
<td>10 9wks.</td>
<td>18 wks.</td>
<td>0 2 5 3</td>
<td></td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10 8wks.</td>
<td>10 8wks.</td>
<td>15 wks.</td>
<td>0 0 4 6</td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 8wks.</td>
<td>10 8wks.</td>
<td>22 wks.</td>
<td>0 0 5 5</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10 9wks.</td>
<td>10 9wks.</td>
<td>23 wks.</td>
<td>0 0 0 10</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 0</td>
<td>14 24</td>
<td></td>
<td></td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>

The comparative results between a single and double vaccination of pigs from the four selected herds in which single-vaccinated animals did not develop satisfactory immunity are shown in Table V. Of 40 pigs, 10 from each herd, given a single vaccination and exposed to HC virus from seven to 14 weeks later, two had a slight reaction, 14 had a severe reaction, and 24 died. Of 40 pigs, 10 from each of the same herds, given a second vaccination and exposed to HC virus four weeks later, 39 remained normal and one had a severe reaction. The percentage of protection for pigs in herds one, two, three, and four exposed to HC virus seven to 14 weeks after a single vaccination was 29 percent, 22 percent, 19 percent and zero percent respectively; four weeks after the second vaccination, the percentage of protection was 100 percent, 100 percent, 100 percent and 96 percent respectively. A bacteriological examination revealed *Pasteurella spp.* in the test pigs that died.

### DISCUSSION

The numerical system of evaluating the reaction of a pig after exposure to HC virus is arbitrary and was developed in an effort to analyze more effectively the degree of immunity induced by vaccination, other than on a "live or dead" basis. It does require the observer to establish in his mind a consistent relationship between the one, two, or three points assigned to a pig each day. When arriving at the percentage of protection, which was based on the calculated percentage of reaction range for each
pig, the lowest percentage range figure was used instead of a mean. This was done to assure that a conservative evaluation would be made on the efficacy of CVG vaccine.

In totaling the number of points for each pig to classify the reaction as either slight, moderate, or severe, there was a variation of 10 points between each classification. For this reason the margin for placement of a pig under these headings was not always necessarily great. Pigs scoring from one to 10 points, however, were seldom sick over four days and quickly returned to normal without appreciable weight loss. These pigs also could be released at the end of two weeks; the same time as those that had no reaction. Therefore, these pigs were included with the nonreacting pigs as having good protection.

The evaluation of vaccinated pigs into categories of good, fair, poor, and no protection, is helpful in describing the relative efficacy of CVG vaccine. It is believed, however, that the over-all evaluation for each one-, three-, and six-month group of pigs expressed in percentage of protection is a more realistic figure. This is because the assigned percentage value for each normal, reacting, and dead pig is added together and averaged; thus a single figure reflects an evaluation of all vaccinated pigs in a group.

A comparison of the percentage of pigs which are classified each year in the categories of normal, slight, moderate, severe, and dead is significant in that it gives some evidence of the immune response in a herd of pigs after vaccination with CVG. The percentage of pigs that survived and conversely the percentage of pigs that died was fairly constant for each of four years. The percentage of pigs that died one month after vaccination was greatest and the percentage that died six months after vaccination was smallest. These percentages indicate that a certain number of pigs do not develop immunity one month after vaccination and that a small percentage of this number develop enough protection in three to six months so they do not die but show reactions when exposed to hog cholera virus.

Of the pigs that survived the greater percentage occurred in the normal category and the percentages in the categories of slight, moderate and severe are respectively less. As the pigs grew older the percentages in the normal and slight categories became progressively less so that at three months after vaccination the percentage of normal dropped by approximately 10 percent and the slight category dropped three to five percent. The drop in percentage of normals and slight category as the pigs grew older is reflected as an increase in the percentage of pigs in the three- and six-month vaccinates of the moderate and severe category. This shift in percentage of the pigs of each category indicates that the resistance to hog cholera infection as measured by sickness becomes less in some pigs as they grow older at six months after vaccination. More than 50 percent of the pigs had good protection; also, the protection was sufficient in the other pigs to prevent death, although they had severe reactions. Based on this data, approximately 50 percent of a herd vaccinated with CVG and exposed to hog cholera would have very little or no reaction. Approximately 13 percent would die and 37 percent would have varying degrees of reaction.
The importance of survival of vaccinated pigs after challenge, however, should not be underestimated when considering the efficacy of CVG vaccine. Even though the reactions were severe in many instances, with an accompanying temporary physical setback, the animals were not a complete economic loss as would be the case if they had died. The average survival for the five years was 87.65 percent and was not lower than 80 percent in any one year.

There are factors other than the degree of immunity which can affect the ability of the pig to survive challenge. Bacterial or viral agents, which apparently did not invade the body of the animal when normal, did invade the body when the animal was infected with HC virus. This was illustrated by the high percentage of isolations of Pasteurella and Salmonella organisms from dead test pigs, and the experience with the bloody diarrhea that occurred in the test pigs in 1958. In addition, Salmonella spp. was isolated from the organs of cholera-infected pigs but from only the intestinal tract of cholera-free litter mates.

Although there was no evidence of naturally occurring hog cholera in CVG-vaccinated pigs on any of the premises, it was recognized that positive evidence of herd exposure to hog cholera could not be obtained under farm conditions unless nonvaccinated controls were left in the herds. This, however, we were unable to do. Nevertheless, it is believed that the opportunity for vaccinated herds to be exposed did exist because of known outbreaks of hog cholera in the same area, as well as on four farm herds adjacent to herds that were vaccinated with CVG vaccine.

The reason for the superiority of double over single vaccination of farm pigs in the four selected herds cannot be satisfactorily explained. It is possible that the high level of protection induced in the double-vaccinated pigs was the result of a second vaccination when the pigs were older and not necessarily the result of a booster effect alone. Conversely, the low level of protection manifested by the single-vaccinated weanling pigs could result from subclinical infection by Pasteurella spp. when vaccinated and when exposed to HC virus. From the standpoint of experimental design, the comparison would have been strengthened if the double-vaccinated pigs had been exposed to HC virus the same number of weeks after vaccination as the single-vaccinated pigs but it was not possible to have single-vaccinated pigs of the proper age available.

The hypothesis that Pasteurella spp. interfered with response of pigs to a single injection of CVG can be supported by the following facts:

1. The four or eight single-vaccinated pigs (depending on the number of farrowing in a year) tested each year for five years had severe reactions or died after exposure to hog cholera virus.

2. Pasteurella was isolated each year from the test pigs that died after exposure to hog cholera.

3. Pasteurella was isolated from the single-vaccinated pigs that died before the herds were double-vaccinated.

4. None of the double-vaccinated pigs died after exposure to hog cholera virus and only one pig had a reaction.

5. It has been proved by experiment that Pasteurella and Salmonella may interfere with the protection of pigs by hog cholera vaccines.
SUMMARY

Crystal Violet Glycerol (CVG) vaccine was used to vaccinate 67,058 farm pigs from 1956 to 1960. Two test pigs from each herd were brought to the Federal Hog Cholera Research Station at one, three, and six months after vaccination and exposed to 1,000,000 MLD of virulent hog cholera (HC) virus under controlled conditions. A total of 2,931 pigs or 4.37 percent of all pigs vaccinated were exposed to HC virus.

The percentage of survival for the one-, three-, and six-month vaccinates was 86.33, 87.80, and 90.78 percent respectively.

Of 1,236 pigs tested one month after vaccination, 60.18 percent had good protection, 15.53 percent had fair protection, 10.51 percent had poor protection, and 13.67 percent had no protection.

Of 1,229 pigs tested three months after vaccination, 49.71 percent had good protection, 19.60 percent had fair protection, 18.22 percent had poor protection, and 12.20 percent had no protection.

Of 466 pigs tested six months after vaccination, 44.63 percent had good protection, 23.39 percent had fair protection, 22.74 percent had poor protection, and 9.22 percent had no protection.

The percentage of protection of the one-, three-, and six-month vaccinates, based on their reaction to HC virus, was 71.91, 67.81 and 67.27 percent respectively.

Subclinical infection with Pasteurella spp. or Salmonella cholera suis is believed to have adversely affected the ability of pigs to withstand exposure to HC virus. An unknown agent responsible for a bloody diarrhea also interfered with the ability of pigs to withstand exposure to HC virus.

Hog cholera did not occur in any farm herd vaccinated with CVG vaccine. Hog cholera was known to have occurred, however, on many farms in the same area and on four farms adjacent to farms where CVG-vaccinated pigs were being raised.

Double vaccination with CVG vaccine induced over 95 percent protection in pigs in four herds where a single vaccination induced less than 30 percent protection.

REFERENCES

SUMMARY AND FINAL REPORT OF THE EXPERIMENT
ON THE ERADICATION OF HOG CHOLERA
IN THE FLORIDA PILOT TEST AREA

M. R. Zinober, D.V.M., and L. O. Mott, D.V.M.*

INTRODUCTION

The Florida pilot test area for the eradication of hog cholera was designated as Suwannee County, Florida, by the United States Department of Agriculture in 1955, in cooperation with the Florida Livestock Board now the Florida Division of Animal Industry. During 1956, the United States Department of Agriculture Hog Cholera Research Station was established at Live Oak, Florida, in Suwannee County where a cooperative Federal-State experimental program was started. Three types of modified live virus vaccines—lapine, porcine, or tissue culture origin—were used for vaccination in combination with a minimum of 15 ml. of hyperimmune anti-hog cholera serum. Close supervision and detailed records of vaccination were started September 1, 1956, and continued 82 months through June 30, 1963. The virulent virus challenge phase of the program, to measure the immunity level of the vaccinates, was started in April, 1957, and was terminated December 31, 1962, a period of 69 months. The confirmation of suspected hog cholera infection by pig inoculation tests as well as investigation of sources of infection was started July 1, 1958, and has continued for 60 months through June 30, 1963. Periodic reports of progress have been made to this association in 1958, 1959, 1960, and 1962. This paper is a summary of the previous reports including the final data developed in fiscal year 1963, which terminates these phases of the pilot plant experiment. A new cooperative project for investigating the spreading characteristics of modified live virus vaccines is presently under way in Suwannee County.

The purposes for the establishment of the program were: (1) to determine the immunogenic efficacy and other characteristics of modified live virus vaccines when used with a minimum of 15 ml. of hog cholera hyperimmune serum, and (2) to determine the effectiveness of a program


The authors wish to thank Dr. Clarence L. Campbell, Jr., Florida State Veterinarian, and the Florida Division of Animal Industry through whose cooperation and assistance this pilot test was made possible. We also wish to acknowledge the assistance of Dr. M. Marmesh (resigned) and Dr. Siebert L. Berlin (resigned), Animal Disease and Parasite Research Division, Dr. Milton J. Tillery and Dr. W. C. Stewart, Animal Disease Eradication Division, given in conducting the work previously presented and published by this association, 1958, 1959, 1960, 1962 as well as this report.
for the eradication of hog cholera from the county as a result of a controlled vaccination program in association with the normal enforcement of Florida state regulations.

VACCINATION

Vaccination with lapine origin, porcine origin, or tissue culture vaccine, with a minimum of 15 ml. of hog cholera hyperimmune serum, were used in fairly equal amounts throughout the period as shown in Table I. The three types of vaccine were from 105 serials from 11 licensees. Thirty-two serials of serum were used throughout the test period. Swine vaccination coverage varied from 70.9 percent in fiscal year 1960 to 76.7 percent in fiscal year 1963, Table I.

CHALLENGE

Challenge consisted of virulent virus exposure with one ml. of virus for each of two pigs from each vaccinated herd at market age, described in the 1958 report. The challenge virus used throughout this experiment consisted of three different lots. Each lot was prepared one pig passage away from BAI lyophilized virus 7183 (1946) and contained not less than 20,000 MLD50 per ml.

Challenge results in percentages of pigs adequately protected and percentages of pigs surviving are given by fiscal years in Table II and illustrated in Graph 1.

It should be noted that the recovery of immunogenic efficacy of lapine origin and porcine origin vaccines in fiscal year 1962, as compared to fiscal years 1960 and 1961, continued in fiscal year 1963. Tissue culture vaccine decreased in immunogenic efficacy in fiscal year 1962 and increased slightly in fiscal year 1963.

**TABLE I**

Vaccination With Hog Cholera Modified Live Virus Vaccine and Estimated Swine Vaccination Coverage in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Lapine Origin</th>
<th>Porcine Origin</th>
<th>Tissue Culture</th>
<th>Total</th>
<th>Estimated Swine Population</th>
<th>Estimated Percent Swine Vaccination Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pigs</td>
<td>Percent of Total</td>
<td>Number of Pigs</td>
<td>Percent of Total</td>
<td>Number of Pigs</td>
<td>Percent of Total</td>
</tr>
<tr>
<td>1957</td>
<td>11550</td>
<td>38.1</td>
<td>10324</td>
<td>34.0</td>
<td>8464</td>
<td>27.9</td>
</tr>
<tr>
<td>1958</td>
<td>8392</td>
<td>27.5</td>
<td>12472</td>
<td>40.8</td>
<td>9702</td>
<td>31.7</td>
</tr>
<tr>
<td>1959</td>
<td>13239</td>
<td>40.2</td>
<td>9439</td>
<td>28.7</td>
<td>10256</td>
<td>31.1</td>
</tr>
<tr>
<td>1960</td>
<td>11267</td>
<td>36.2</td>
<td>12951</td>
<td>41.6</td>
<td>6681</td>
<td>22.1</td>
</tr>
<tr>
<td>1961</td>
<td>10619</td>
<td>35.0</td>
<td>11851</td>
<td>38.4</td>
<td>8202</td>
<td>26.6</td>
</tr>
<tr>
<td>1962</td>
<td>9307</td>
<td>31.2</td>
<td>10931</td>
<td>36.6</td>
<td>9622</td>
<td>32.2</td>
</tr>
<tr>
<td>1963</td>
<td>14106</td>
<td>43.2</td>
<td>10947</td>
<td>33.5</td>
<td>7584</td>
<td>23.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>78680</strong></td>
<td><strong>36.0</strong></td>
<td><strong>78915</strong></td>
<td><strong>36.1</strong></td>
<td><strong>60711</strong></td>
<td><strong>27.8</strong></td>
</tr>
</tbody>
</table>
Graph 1. Challenge of Vaccinated Pigs in Suwannee County, Florida
<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Lapine Origin</th>
<th>Porcine Origin</th>
<th>Tissue Culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pigs Challenged</td>
<td>Percent Adequately Protected\textsuperscript{a}</td>
<td>Percent Survived</td>
<td>Number of Pigs Challenged</td>
</tr>
<tr>
<td>1957\textsuperscript{b}</td>
<td>67</td>
<td>97.0</td>
<td>97.0</td>
<td>83</td>
</tr>
<tr>
<td>1958</td>
<td>415</td>
<td>93.7</td>
<td>95.7</td>
<td>388</td>
</tr>
<tr>
<td>1959</td>
<td>168</td>
<td>81.5</td>
<td>82.7</td>
<td>215</td>
</tr>
<tr>
<td>1960</td>
<td>358</td>
<td>86.9</td>
<td>91.9</td>
<td>237</td>
</tr>
<tr>
<td>1961</td>
<td>230</td>
<td>84.3</td>
<td>90.4</td>
<td>221</td>
</tr>
<tr>
<td>1962</td>
<td>295</td>
<td>88.1</td>
<td>92.9</td>
<td>321</td>
</tr>
<tr>
<td>1963\textsuperscript{c}</td>
<td>169</td>
<td>91.1</td>
<td>95.3</td>
<td>242</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1702</td>
<td>88.7</td>
<td>92.4</td>
<td>1707</td>
</tr>
</tbody>
</table>

\textsuperscript{a}For determination of adequate protection see Zinober and Marmesh.\textsuperscript{1}

\textsuperscript{b}Last three months only.

\textsuperscript{c}First six months only.
TABLE III
Factors Influencing Challenge Results (69 Months) in Suwannee County, Florida
1. Farm Variation

<table>
<thead>
<tr>
<th>Percent of Adequately Protected Pigs</th>
<th>Number of Pigs Challenged</th>
<th>Number of Pigs Adequately Protected</th>
<th>Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-100 (Average 92.2)</td>
<td>4012</td>
<td>3699</td>
<td>455</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Average 8.8 pigs per farm) 80.7</td>
</tr>
<tr>
<td>0-79 (Average 66.5)</td>
<td>830</td>
<td>552</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Average 7.6 pigs per farm) 19.3</td>
</tr>
<tr>
<td>Total</td>
<td>4842</td>
<td>4251</td>
<td>564</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

*For determination of adequate protection see Zinober and Marmesh.*

TABLE IV
Factors Influencing Challenge Results in Suwannee County, Florida
2. Number of Days Between Vaccination and Challenge

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Number of Days Between Vaccination and Challenge</th>
<th>Number of Days Between Vaccination and Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 - 359</td>
<td>360 - 1410</td>
</tr>
<tr>
<td></td>
<td>Number of Pigs Challenged</td>
<td>Percent Adequately Protected</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>1490</td>
<td>87.6</td>
</tr>
<tr>
<td>Porcine Origin</td>
<td>1561</td>
<td>83.6</td>
</tr>
<tr>
<td>Total</td>
<td>3051</td>
<td>85.5</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>1372</td>
<td>90.7</td>
</tr>
</tbody>
</table>

*For determination of adequate protection see Zinober and Marmesh.*

Data have been collected on various factors which were thought to have some influence on the development of immunity. Five of these factors are discussed:

1. The effect of farm variation on development of immunity is shown in Table III. All pigs challenged during the six-year test period for each of 564 swine owners on farms were totaled. The farms were then divided into those having adequately or inadequately protected pigs in accordance with authors' report for 1958. The results show 66.5 percent average adequate protection for challenged pigs in 109 or 19.3 percent of farms with inadequately protected pigs; this compares with 92.2 percent average
adequate protection for challenged pigs on 455 or 80.7 percent of farms with adequately protected pigs. The significance of the 25.7 percent less adequately protected pigs on 19.3 percent of the test area farms is that it appears to be due to some unidentified condition which warrants further investigation.

2. The result of studies to determine the effect of length of post-vaccination time on reaction to challenge was reported in 1959. This study has been continued and extended. The results are shown in Table IV. These data confirm the phenomenon studied earlier—that with an increase of post-vaccination time there is an increase of percentage of protected pigs. Pigs vaccinated with lapine origin or porcine origin vaccines, when challenged more than 360 days post-vaccination, showed 7.8 percent more adequately protected pigs than pigs challenged less than 360 days post-vaccination. Pigs vaccinated with tissue culture vaccine showed no significant difference.

Several hypotheses have been advanced in explanation of this seemingly anomalous phenomenon. These will be tested and reported on in the near future. One of the most likely of these is that the challenged pigs received continued secondary exposure to live vaccine virus eliminated by vaccinated pigs on the farm. Torrey et al. have demonstrated the transmission by contact of immunizing virus from lapine-origin and porcine-origin, modified live virus-vaccinated pigs to susceptible pigs. We have confirmed this in our laboratory. This eliminated vaccine virus could have acted as a booster viral antigen stimulating the defense mechanism of the already vaccinated pig to increase its immunity.

3. Another factor is the quantity of serum used at time of vaccination. The effect of large, repeated doses of serum prior to vaccination has been described by Kovalenko, Dunne and Alibasoglu, and Dunne and Kradel. We have compared the effect of variable doses of serum administered simultaneously with the vaccine. These data are given in Table V. During fiscal years 1957, 1958 and 1959, there was no significant difference in percentage of protected pigs regardless of the amount of serum administered simultaneously with the vaccine. Beginning in fiscal year 1960, there was a significant difference in the percentage of protected pigs depending on whether they received 15-19 ml. of serum or 20 ml. or more of serum. The larger dose of serum in every case caused a significant decrease in the percentage of protected pigs. This tendency was true of all vaccine types and for each of the last four fiscal years. It is significant that in November, 1960, as the result of a change in procurement regulations by the Florida Division of Animal Industry, fresher vaccines were used in the pilot hog cholera eradication area. The results of our study as shown in Table V seem to indicate that the administration of 20 ml. or more of serum simultaneously with vaccine protected less pigs than when the serum dose was less than 20 ml.

4. A six-year study of the effects of stress factors on immunity development was made by selecting the challenge results on 206 herds reported as having stress factors at the time of vaccination. The data
TABLE V
Factors Influencing Challenge Results in Suwannee County, Florida
3. Serum Dosage Administered at Vaccination

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Type of Vaccine and Serum Dosage</th>
<th>Lapine Origin</th>
<th>Porcine Origin</th>
<th>Tissue Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-19 ml.</td>
<td>20+ ml.</td>
<td>15-19 ml.</td>
<td>20+ ml.</td>
</tr>
<tr>
<td></td>
<td>Number of Pigs Challenged</td>
<td>Percent Adequately Protected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Number of Pigs Challenged</td>
<td>Percent Adequately Protected&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1957&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14</td>
<td>92.9</td>
<td>19</td>
<td>94.7</td>
</tr>
<tr>
<td>1958</td>
<td>249</td>
<td>88.0</td>
<td>119</td>
<td>86.6</td>
</tr>
<tr>
<td>1959</td>
<td>139</td>
<td>81.3</td>
<td>15</td>
<td>80.0</td>
</tr>
<tr>
<td>Total</td>
<td>402</td>
<td>85.8</td>
<td>153</td>
<td>86.9</td>
</tr>
<tr>
<td>1960</td>
<td>278</td>
<td>87.4</td>
<td>25</td>
<td>80.0</td>
</tr>
<tr>
<td>1961</td>
<td>185</td>
<td>87.6</td>
<td>39</td>
<td>74.4</td>
</tr>
<tr>
<td>1962</td>
<td>205</td>
<td>89.3</td>
<td>108</td>
<td>78.7</td>
</tr>
<tr>
<td>1963&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55</td>
<td>94.5</td>
<td>108</td>
<td>83.3</td>
</tr>
<tr>
<td>Total</td>
<td>723</td>
<td>87.1</td>
<td>280</td>
<td>81.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>For determination of adequate protection see Zinober and Marmesh.<sup>1</sup>

<sup>b</sup>Last three months only.

<sup>c</sup>First six months only.
showed 354 pigs or 85.9 percent of 412 pigs challenged from these herds were adequately protected, which is similar to the overall 87.8 percent of pigs protected, Table II. These results indicate stress factors in herds at the time of vaccination did not have an adverse effect on immunity development.

5. The most significant factor found to have an adverse effect on the percentage of adequately protected pigs was the old age of modified live virus vaccines when used for vaccination. This finding was reported to this association in 1960,3 and in 1962.4 The inverse relationship between average age of vaccine at time of use and immunogenicity of the vaccine was reported. The highest percentage of protected pigs by all vaccines combined for a 12-month period ending April 30, 1958 was 95.3 percent. This was during the early part of the field trial, when purchased vaccines were used fresh. The lowest percentage of protected pigs by all vaccines for a 12-month period was 75.3 percent for the period ending January 31, 1961. The period of decline in immunity was directly associated with the use of vaccines of older age. As a result of these findings, the Florida Division of Animal Industry in November, 1960, made a change in procurement regulations, from which date fresher vaccines were used. About six months following the change to fresher vaccines, the vaccinated pigs receiving these products appeared at the market from which vaccinated pigs are selected for challenge. The results of such challenge showed the decline in immunity had stopped. Since that time there has been a gradual increase in the percentage of all pigs protected for each fiscal year to 91.9 percent for fiscal year 1963. (See Table II and Graph 1.)

Porcine origin vaccine, which showed the greatest decline in percentage of pigs protected for a 12-month period from 97.2 percent on May 31, 1958, to 57.5 percent on December 31, 1960, has also showed the greatest recovery in immunogenic efficacy to 95.9 percent in fiscal year 1963.

Lapine origin vaccine showed the same general pattern of decline from 97 percent in fiscal year 1957 to 81.5 percent in fiscal year 1959 and recovery to 91.1 percent in fiscal year 1963 of immunogenic efficacy but to a milder degree as compared with porcine origin vaccine.

Tissue culture vaccine has presented a different pattern from the other vaccines as it showed a decline in percentage of pigs protected from 97.9 percent in fiscal year 1957 to 86.8 percent in fiscal year 1959. Following the change to use of fresher vaccines in November, 1960, it showed a slight increase in percentage of pigs protected to 90.5 percent in fiscal year 1960, then again declined and leveled off at an average of about 89 percent for three years through fiscal year 1963.

ERADICATION OF HOG CHOLERA

There have been two 12-month periods of freedom from hog cholera in Suwannee County, although there have been one or more cases reported in each of the last five fiscal years as shown in Table VI. One of the 12-month periods was reported by Campbell9 as occurring in 1960 and 1961. The other 12-month period occurred during 1962 and 1963. A case which
TABLE VI

Confirmed Cases of Hog Cholera in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Vaccinated</th>
<th>Non-Vaccinated</th>
<th>Total</th>
<th>Vaccinated</th>
<th>Non-Vaccinated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td></td>
</tr>
<tr>
<td>1959</td>
<td>3</td>
<td>23.1</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>1960</td>
<td>7</td>
<td>75.0</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>1961</td>
<td>4</td>
<td>84.6</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1962</td>
<td>2</td>
<td>100.0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1963</td>
<td>0</td>
<td>100.0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>63.8</td>
<td>21</td>
<td>14</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>

TABLE VII

Vaccination Status of Swine Delivered for Sale at Suwannee Valley Livestock Market, Live Oak, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Slaughter Hogs</th>
<th>Feeder Pigs</th>
<th>Total Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Hogs</td>
<td>Vaccinated</td>
<td>Non-Vaccinated</td>
</tr>
<tr>
<td>1959</td>
<td>26779</td>
<td>73.0</td>
<td>27.0</td>
</tr>
<tr>
<td>1960</td>
<td>26707</td>
<td>68.7</td>
<td>31.3</td>
</tr>
<tr>
<td>1961</td>
<td>23930</td>
<td>78.2</td>
<td>21.8</td>
</tr>
<tr>
<td>1962</td>
<td>28504</td>
<td>76.6</td>
<td>23.4</td>
</tr>
<tr>
<td>1963</td>
<td>29271</td>
<td>76.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Total</td>
<td>141191</td>
<td>74.8</td>
<td>25.2</td>
</tr>
</tbody>
</table>

was suspected of being hog cholera was reported on May 20, 1963, and was later confirmed. This positive case terminated the latest 12-month period of freedom from hog cholera.

A change in epizootiology of hog cholera in Suwannee County has occurred and is illustrated in Table VI and Graph 2. In fiscal year 1959, 81.3 percent of the confirmed cases were associated with recent purchases of swine from a public market. From fiscal year 1960 to the present, while the use of modified live virus on the farm continued, 76.2 percent of confirmed cases occurred in farm-raised swine.

The public market continues as an important potential disseminating focus of hog cholera. Large numbers of nonvaccinated swine were
Graph 2. Confirmed Cases of Hog Cholera in Suwannee County, Florida
delivered to the public market in Suwannee County during the last five fiscal years as shown in Table VII. The most alarming feature of Table VII is that the greater percentage of nonvaccinated pigs delivered to the public market were among the feeders. These were the same pigs that were taken to farms after having passed through the public market and each one was therefore a potential focus of hog cholera infection. Table VIII shows the large number of nonvaccinated pigs (7399) which were removed from the local public market premises during the last three fiscal years; they were not intended for immediate slaughter and were taken to farms. We consider it extremely fortuitous that there were only two outbreaks in these three years (see Table VI) which were associated with recent public market purchases.

SUMMARY AND CONCLUSIONS

1. Average annual swine vaccination coverage in the pilot hog cholera eradication area for the period from April, 1957, through June, 1963, was 73.1 percent.
2. Following challenge of 4842 pigs, 87.8 percent were adequately protected and 91.2 percent survived.
3. The farm-to-farm variation in the ability of pigs to develop an adequate immunity over the 69-month test period showed an average difference of 25.7 percent of pigs protected between farms with adequately (80 to 100 percent) and inadequately (below 80 percent) protected swine.
4. A greater number of pigs (93.3 percent) which were vaccinated with lapine origin or porcine origin vaccines were protected when they were challenged 360 days or more post-vaccination than were protected when they were challenged less than 360 days post-vaccination (85.5 percent). Pigs vaccinated with tissue culture vaccine showed no significant difference.
ERADICATION OF HOG CHOLERA

5. Twenty ml. or larger doses of anti-hog cholera hyperimmune serum administered simultaneously with modified live virus vaccines of all types resulted in lower percentages of adequately protected pigs than smaller serum doses similarly administered for the different vaccine types used during fiscal years 1960, 1961, 1962, and 1963.

6. Stress factors recorded in 206 herds at time of vaccination had no significant effect on the development of immunity.

7. The most significant factor found to have an adverse effect on the percentage of adequately protected pigs was vaccine age at the time of vaccine administration.

8. Fifty-eight cases of hog cholera were confirmed in fiscal years 1959 to 1963, inclusive, of which 63.8 percent occurred in farm-raised swine.

9. During fiscal years 1959 to 1963, inclusive, more than one half of the feeder pigs (52.8 percent) delivered for sale to the public market were not vaccinated.

10. During fiscal years 1961, 1962, and 1963, 7399 nonvaccinated pigs, not intended for immediate slaughter, were removed from the local public market premises without vaccination and were taken to farms.

REFERENCES


INTRODUCTION

The advancements being made in hog cholera eradication have created new problems and made it necessary to have more specific information about the virus. One of these problems is the lack of satisfactory methods to inactivate the virus in pork and pork products. Heating at a specified temperature and for a specified time that will inactivate hog cholera virus appeared to be a logical procedure, providing it did not make the meat products unmarketable.

Regulations governing meat inspection require all carcasses showing lesions of hog cholera to be condemned. The virus in carcasses from animals in the incubative stage of the disease cannot be detected by gross inspection. Thus, either uncooked or improperly cooked meat scraps from these carcasses in garbage could be a source of infection.

The only requirement in Federal Meat Inspection Regulations1 for heating pork or pork products has been to destroy trichinae (Part 18, Section 18.10, 1959). This regulation states: "...pork muscle tissue shall be heated to a temperature not lower than 137°F..." The regulation, however, does not specify the length of time the meat should be heated. Federal Regulations and Laws of the Animal Disease Eradication Division2 governing swine products derived from swine affected with hog cholera and other communicable swine diseases require "...all swine products to be specially processed shall be heated to an internal temperature of at least 147°F for 30 minutes or to an internal temperature of 156°F momentarily." (Part 76, Sub-chapter C, Section 76.14, September 7, 1962.)

Leresche3 1956 gave the following times and temperatures to inactivate hog cholera virus in sausages: "Bratwurst" 29-31 mm, 80-82°F for 10 minutes; "Vienna" 22-33 mm, smoking at 80°F for 45 min. and scalding 80°F for eight minutes; and "Lyonerwurst" 59-62 mm, smoking at 82-85°F for 50 minutes and scalding at 81-82°F for 45 minutes.

References in the literature to heat inactivation of hog cholera virus are limited and refer mainly to virus inactivation for vaccines and to virus survival. Most reports do not describe the method or the medium for heating the virus. In 1939, Ray and Whipple4 collected data on temperatures in closed compartments of automobiles, such as might be used by practicing veterinarians in swine immunization work. They then heated hog cholera virus in phenolized blood at the same temperatures. They found that
HEAT INACTIVATION OF HOG CHOLERA VIRUS

Phenolized hog-cholera virus heated at 58.5°C (137.3°F) for one hour did not cause hog cholera when injected into seven susceptible pigs. Viruses heated three to four hours at lower temperatures were either inactivated or lost their virulence, but not necessarily their capacity to immunize. Merchant and Packer stated that thermal inactivation occurs by exposure to 55°C for 30 minutes and 60°C for 10 minutes but they did not give the medium in which the virus was heated. They stated that thermal inactivation of hog cholera virus in dried blood occurs at 72°C for one hour.

The 1912 Annual Report of the Chief of the Bureau of Animal Industry reported many experiments attempting to attenuate hog cholera virus in blood. Blood heated to 60°C for 30 minutes did not destroy the virulence of the virus. A second report in 1915 stated that virus in blood heated at 50°C for 12 hours, 55°C for six hours, and 60°C for 1-1/2 hours did not destroy the virus. A third report in 1924 stated that virus was attenuated when heated from 92.3°F to 100.7°F for seven days. The Code of Federal Regulations (Title 9, Part 119.20, April 1959) governing the production of serums for biological use requires heating to 58.5°C for 30 minutes.

The objective of this study was to determine the time and temperature required to inactivate hog cholera virus in swine blood and serum. Defibrinated blood and serum were selected for this part of the study because they form an integral part of all swine products, were readily available, and were easy to measure in any desired amount. These could also be injected after heating without further handling or preparation.

MATERIALS AND METHODS

The virus used in this study was obtained from a second pig passage of virus lyophilized in 1945 at the Animal Disease Station, Beltsville, Maryland. One milliliter of blood contained 10^5 tissue culture infective doses of virus which demonstrated no variant characteristics. The blood containing virus was obtained by bleeding cholera-infected pigs seven days after injection. It was defibrinated mechanically and stored in quantities of 250 ml. in glass bottles at -58°C.

The pigs used to test for virus were obtained by hysterectomy and raised on a colostrum-free diet. They weighed 50 to 60 pounds and while on experiment were kept in individual isolation cages with negative pressure ventilation.

Standard constant temperature water baths with agitating false bottoms and containing 36 liters of water were used to heat the blood and serum. They were equipped with a high and a low input electric unit. A 60 second percent-o-cycle* was used to increase the temperature gradually for a given length of time. Centigrade laboratory thermometers with a temperature range of 10 below to 100 degrees above zero were used for determining temperatures. The thermometers were checked with a certified centigrade thermometer and those selected had a maximum variation of ±1/4°.

Seventeen ml. of blood were placed in a 15 x 125 mm. test tube and the tube closed with a one-hole rubber stopper. This amount of blood completely filled the tube when the bulb of the thermometer was inserted through the stopper to the midpoint of the tube. The test tubes were placed horizontally on a test tube support and submerged in the water bath so that the tubes were in about the central portion of the water. When the heating period was completed, the tube of blood was immersed in an ice water bath until the temperature dropped to five degrees C. The time required to raise the blood to the required temperature was called the preheating time; the time the blood was held at a certain temperature was called the heating time; and the time required to cool the blood to five degrees C was called the chilling time.

A temperature of 60°C for 30 minutes was selected as a starting point. When a sample was heated at a certain temperature, comparable samples were heated two degrees above and two degrees below for the same time to check any variation which might occur. After one set of samples was heated and tested for the presence of virus by animal inoculation, another set of samples was heated at the next higher temperature. After the inactivation temperature for 30 minutes was established, the preheating and heating times were varied to determine how this affected the temperatures necessary for virus inactivation. A total of 49 tests was made to establish the effects of heating at different temperatures for various lengths of time on the virus.

To prepare material for animal inoculation, the sample was stirred with a sterile glass rod, removed from the test tube and placed in a syringe. Five ml. of the sample were injected subcutaneously into each of two cholera-susceptible pigs.

In order to determine the immune status of those pigs not showing symptoms, each was given two ml. of virulent virus at 14 days after the initial inoculation. Each of those pigs that recovered after showing definite symptoms of hog cholera was also challenged with two ml. of virulent virus 14 days after recovery.

Evaluation of the test sample was as follows: when the pigs remained normal, the virus was considered inactivated; when the pigs became sick and died, the virus was not inactivated; when the pigs became sick and recovered and were later resistant to virulent virus, the heated virus was considered attenuated. A postmortem examination was made of each pig that died.

RESULTS

Hog cholera virus in defibrinated blood was inactivated when heated at 69°C for 30 minutes but was not inactivated when heated at 60°C, 62°C, 64°C, 66°C, and 68°C for 30 minutes (Table I).

When the heating time was increased to 45 and 60 minutes, the virus was inactivated at 68°C and 66°C, respectively.

When the preheating time was increased from three to 120 minutes and the heating time kept at 30 minutes, the virus was inactivated at 68°C. It was not inactivated at 66°C when the preheating time was increased to
HEAT INACTIVATION OF HOG CHOLERA VIRUS

Table I

<table>
<thead>
<tr>
<th>Material</th>
<th>Number Trials</th>
<th>Preheating Time (Min.)</th>
<th>Heating Time</th>
<th>Temperature</th>
<th>Chilling Time (Min.)</th>
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<tr>
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<td>64</td>
<td>156.2</td>
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<tr>
<td>80% blood</td>
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<td>30 minutes</td>
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<td>156.2</td>
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<td>Serum</td>
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<td>3</td>
<td>30 minutes</td>
<td>66</td>
<td>156.2</td>
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<tr>
<td>Serum</td>
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<tr>
<td>Serum</td>
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<td>4</td>
<td>Momentarily</td>
<td>97</td>
<td>206.6</td>
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</tbody>
</table>

140 minutes. Blood containing virus when heated to 97°C or boiling and then immediately cooled was not inactivated.

Virus in blood diluted to 80 percent with physiological saline (0.85 percent NaCl) was not inactivated at 68°C for 30 minutes.

Virus in serum was attenuated when heated to 68°C for 30 minutes and inactivated when heated to boiling momentarily at 97°C.

DISCUSSION

When blood was heated to 60°C or above, it coagulated to a firm consistency and became a chocolate color. As the temperature and time were increased, the blood became more firm.

The temperature at which hog cholera virus was inactivated was influenced by the medium containing the virus, the preheating time and the heating time. Changing any one of these factors influenced the effect of the others. At 68°C for 30 minutes, an increase of the preheating time from three to 120 minutes was effective in inactivating the virus. When the preheating time was three minutes and the heating time was increased from...
30 minutes to 60 minutes, the virus was inactivated at 66°C. An increase in the heating time from 30 to 45 minutes inactivated the virus at 68°C. Sixty-nine degrees for 30 minutes was the critical temperature for hog cholera virus when carried in defibrinated blood. Below 69°C the preheating time must be increased or the heating time increased above 30 minutes to inactivate the virus.

Heat-coagulated blood provided protection for the virus because the time and temperature required to attenuate or inactivate the virus in clear serum did not inactivate the virus when contained in defibrinated blood.

Experimental work will be continued to establish temperatures and times that will inactivate hog cholera virus in commercial-type pork and pork products.

SUMMARY

Hog cholera virus contained in defibrinated swine blood was inactivated when heated to 69°C for 30 minutes. It was also inactivated at 68°C for 45 minutes and 66°C for 60 minutes.

Blood diluted to 80 percent with physiological saline was not inactivated when heated at 68°C for 30 minutes.

Hog cholera virus in swine serum was attenuated at 68°C for 30 minutes. Heating virus-serum to boiling temperatures momentarily, however, did inactivate the virus.

REFERENCES

STATUS OF STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

G. H. Wise, D.V.M.*

The past year has seen initiation of a cooperative effort to eradicate hog cholera from the United States. This program, long desired and worked for by this Association and other groups, is based upon principles adopted in 1956 by your Committee on the Nationwide Eradication of Hog Cholera. Last year these principles were utilized in developing a four-phase program, which the Committee recommended be used as a basis for State-Federal program activity. The Animal Disease Eradication Division accepted this recommendation and has adhered to the four-phase program in working with the various States.

PROGRESS BY STATES

Results of this cooperative activity are shown in Figure 1.

Figure 1

*Chief Staff Officer, Hog Cholera Eradication, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
In less than 12 months, (December 1962 through September 1963), 36 States and Puerto Rico formally entered the cooperative program through the procedures agreed upon between this Association and the Animal Disease Eradication Division. Of these, 24 are operating in the initial, or preparatory, phase, eight have advanced to Phase II, one is in Phase III, and three are in Phase IV. Seventy-two percent of the swine population is included in States in the program, and over 30 percent of the total swine population is included in States that have advanced beyond the first phase of the program.

This rapid progress is strong evidence of the wide support for this cooperative effort and the recommended principles and procedures for carrying out the campaign.

REPORTING AND INVESTIGATION

The prompt reporting of hog cholera outbreaks is essential in the conduct of this program. Associated with prompt reporting is intensive epizootiological study by regulatory personnel. Both of these points are included in the four-phase program, and are in full operation in all States having advanced beyond Phase I.

![Hog Cholera Eradication](chart.png)

*Includes Puerto Rico

Figure 2
Figure 2 illustrates changes in reporting systems. A survey conducted by the Animal Disease Eradication Division in the spring of 1962 indicated that only 20 percent of the States had an established system for the immediate reporting of hog cholera outbreaks by telephone, telegraph, or equally rapid means. By July 1963, 72 percent of the States participating in the cooperative program had established systems for immediate reporting, although only 13 percent of the remaining States had done so.

### Hog Cholera Eradication

#### OUTBREAK INVESTIGATIONS

*Traced to Source*

<table>
<thead>
<tr>
<th>PHASE II AND III STATES</th>
<th>58%</th>
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<tr>
<td>PHASE I STATES</td>
<td>44%</td>
</tr>
<tr>
<td>OTHER STATES</td>
<td>24%</td>
</tr>
</tbody>
</table>

*July-August 1963*

Figure 3 shows the relative success of completed outbreak investigations for July and August 1963, the first months for which detailed reports by State of completed investigations were available.

States operating in Phases II, III or IV of the program carry out investigations of all reported outbreaks. During July and August results of completed investigations in these States indicated that 58 percent of the cases the investigation was successful in establishing a probable source of infection. For States participating in Phase I the figure was 44 percent, and for the remaining States only 24 percent.

During July and August completed investigations indicated that infection was most commonly introduced through the movement of swine, with 36 percent due to intrastate movement, and four percent to interstate movement.
During the past year there has been increased emphasis on garbage feeding premise inspections. Results are demonstrated in Figure 4. In June 1962, there were 8,742 such premises reported, with 75 percent receiving inspection once a month. Figures for twice a month inspections were not being reported at that time. In July 1963, many additional premises had been located, so that 9,782 were reported, with 85 percent being inspected once and 53 percent twice.

It is obvious that this increase both in premises under inspection and in level of inspection reflects increased interest and increased personnel demands for this activity. Continued support, both in personnel and in enforcement of laws and regulations, will be needed not only to complete the eradication of hog cholera, but as a bulwark against the introduction of exotic diseases and added protection against such constant threats as tuberculosis, salmonellosis, and trichinosis.

INCIDENCE OF HOG CHOLERA

During Fiscal Year 1963 hog cholera was reported from 685 counties in 40 States and Puerto Rico (Figure 5) as compared to more than 1,000
counties in 42 States during Fiscal Year 1962. Reports by quarter for the last five fiscal years are shown in Figure 6.

While these show a marked decline in reported outbreaks during the past year it must not be assumed that this represents an accurate index of the number of cases occurring. It is possible that this decline does indicate a lower relative incidence as compared to previous years. If so, it is encouraging that this took place during the same period that cooperative program procedures were being put in effect.

We must remember, however, that this shows us only that part of the hog cholera "iceberg" that is visible above the water line. Program activity in reporting and investigation can be expected to strengthen our knowledge of the total incidence, but at this point we cannot project what the volume of the unreported infection may be.

INTERSTATE MOVEMENTS

Among the items considered by this Association last year was standards for livestock markets approved under Federal regulations to handle swine in interstate commerce. Through August 1963, 762 markets had been approved in 33 States. Of these, 488 were approved to handle all classes of swine, 50 to handle feeding and breeding swine, and 224 to receive only slaughter swine.
During Fiscal Year 1963 total sales of hog cholera vaccines increased over the previous year. This is the third successive year in which such an increase has been noted, so that in Fiscal Year 1963 vaccine sales were 40 percent higher than in Fiscal Year 1960. As there has been no comparable increase in swine population during this same period it would appear that the attention devoted to publicizing the need for increased vaccination may have shown results.

Of significant assistance in evaluating this aspect of the program has been the cooperation of vaccine manufacturers and distributors in supplying information on the sales of hog cholera vaccines by States. The first report, covering the last quarter of Fiscal Year 1963, has been compiled and distributed by the Animal Inspection and Quarantine Division. As successive reports are rendered it will be possible in each State to evaluate the level of hog cholera vaccination and the comparative use of the general types of products.
The accepted principles for hog cholera eradication are embodied in the phased program approach, designed to contain the disease, reduce incidence, and finally to eliminate outbreaks as they occur. Results to date indicate the soundness of this approach.

By the end of Fiscal Year 1964, it is anticipated that all States should be included under the program, with 15 additional States being added to Phase II activity, and an additional 10 States moving into Phase III or IV.

The goals are felt to be realistic in view of progress during the past year. Undiminished interest and support will be necessary if they are to be achieved.

**COMMENTS**

Accomplishments in this first year of the cooperative hog cholera eradication program have been many, and are impressive as illustrations of progress within the framework of established program procedures. The overall picture seems to illustrate that these procedures are sound, and, if conscientiously carried out, can lead to final eradication.

It would be foolish to assume, however, that we have all the answers to all the problems, or that our present procedures have been sufficiently tried to explore possible faults. We are beyond the stage of speculation on how to initiate the program and are engaged in applying our present knowledge to conduct the program. As this knowledge grows, we should critically examine our opinions and predictions and be unwilling to accept those not based on reasonable evidence.

In looking to the future the goal of eradication must be the criterion for determining action. Due to the long existence of this disease, it is often easy to confuse improved control with final eradication. It cannot be expected that eradication will be accomplished without changes in attitudes and practices.

In several States, including some with little or no reported incidence of hog cholera, the opinion is expressed that eradication can be carried out by voluntary removal of infected and exposed swine. Our previous experience indicates that successful eradication of livestock disease in this country has usually involved condemnation in the final stages, normally with payment of indemnity. It would appear questionable, in light of this experience, to base all plans for successful eradication in any State on measures short of authority to condemn animals. It may be that such steps will not be necessary in some States, but lack of standby authority and preparation for such action could seriously impede the program if this final action is necessary to wipe out pockets of infection.

While much has been done to provide uniformity of approach to this program, particularly in field procedures, it appears that the old question of reasonable uniformity between States on the matter of importation requirements is not entirely answered. Variation in industry patterns and traditions can be expected to result in some differences in these
requirements, but in thinking of the goal of eradication the need to consider how best to accomplish this goal must be paramount, rather than what might be called a "business as usual" approach.

We have made a beginning—perhaps a significant one. Time, support, and action based on knowledge, will demonstrate how rapidly this beginning will expand to complete elimination of hog cholera.
It is extremely gratifying to report that this past year has seen the inauguration of a concerted action program directed toward the eradication of hog cholera. Since this body last convened the Animal Disease Eradication Division, cooperating with the various states, has formally entered into cooperative hog cholera programs in 36 of the 50 states. Of this number, 24 are engaged in Phase I, the initial or preparatory phase; eight are in Phase II, Reduction of Incidence; one in Phase III, Elimination of Outbreaks, and three have advanced to Phase IV, Protection Against Reinfection.

It has been observed that in these states wherein they have embarked upon a program as recommended in the last year's Committee Report, that for the most part the standards are workable and successful when conscientiously applied, and have resulted in marked improvement in such basic areas as prompt reporting of suspected cases, investigations of outbreaks, and increasing the level of swine vaccinated.

In view of the steady progression of three states in their approach to hog cholera free status, beyond the contemplation of this Committee in its original deliberations developing recommendations for movement of swine into free areas, the Committee has been confronted with the inability of these states to obtain swine vaccinated with inactivated vaccine sufficient to meet their needs. The Committee engaged in deliberations in which it was requested that swine treated with modified live virus vaccines be allowed entry into such areas with isolation and inspection at destination in order to meet the normal import demands. After due consideration your Committee reaffirms its position that the use of modified live virus vaccines in a hog cholera free area, or the importation of swine recently vaccinated with such products would be a retrogression; however, in meeting this problem we feel that the following recommendation can be adopted as a practical answer without compromising this high level of attainment. Therefore, your Committee recommends that that section of the recommendation for establishment of hog cholera free areas concerning the importation of swine be amended to read as follows:

**Importation of Swine:**

a. Importation for purposes other than immediate slaughter must be under permit and either:
(1) Originate in an adjacent hog cholera free state and enter on a veterinary health certificate, such swine to be held in strict isolation and quarantine upon arrival at destination for a period of at least 21 days, with inspection and freedom from disease at the end of this period; or

(2) Have been vaccinated with inactivated (killed) vaccine not less than 21 days nor more than six months prior to shipment and move on veterinary health certificate, with isolation and quarantine upon arrival at destination for at least 21 days with inspection and freedom from disease at the end of this period; or

(3) Originate in a state having an immediate hog cholera reporting system on a farm of origin where such swine were born and such farm is not used to assemble, buy or sell swine brought in from other sources, with all swine on such farm at time of shipment to have been located there for not less than 21 days, and that hog cholera has not been known to exist on such farm or on adjoining farms for a period of one year prior thereto. The swine shipped shall move without contact with other swine or facilities used by other swine en route, enter on a veterinary health certificate, and be held in strict isolation and quarantine upon arrival at destination for at least 21 days, with inspection and freedom from disease at the end of this period.

b. All swine imported shall be transported in vehicles cleaned and disinfected and bedded under supervision just prior to shipment.

The Committee heard requests that it consider recommending a revision of federal interstate regulations to require the vaccination of feeder pigs moved interstate from approved and non-approved markets. It is our opinion following extended deliberations on this subject that this Association, having adopted the 1961 Report of this Committee which emphasized proper identification and pre-movement vaccination of swine in interstate commerce, request the Secretary's Hog Cholera Advisory Committee to hammer out the details in resolving the problem of interstate shipments so as to best serve the interests of hog cholera eradication, rather than those of special interest groups.

It was brought to the attention of the Committee that maintaining bi-monthly inspections of garbage feeding establishments creates economic burdens on certain states, and it was recommended that the standards proposed by this Committee for inspection of such establishments be revised so as to permit a lesser amount of supervision in establishments which are evidencing complete cooperation in their cooking activities, and more frequent inspections for borderline and known violators. In view of the concerted efforts which through the years have been directed toward the development of a bi-monthly inspection system, and the apparent resultant success of such a system, your Committee feels at this time it would be unwise to amend that provision which requires bi-monthly inspection of licensed garbage feeding establishments.
The Committee was privileged to have as a participant in its discussions a representative of Livestock Conservation, Inc., and we wish to commend that group for recommendations recently adopted directed toward the national hog cholera eradication effort. Many of these recommendations have heretofore been considered and approved by this Association. One point made by the LCI Committee which has been previously discussed by our group but not heretofore recommended is that of compulsory vaccination of all garbage fed swine as protection against outbreaks that endanger surrounding herds. In this respect, your Committee recommends that the states give consideration to requiring compulsory vaccination of garbage fed swine until such time as they attain a hog cholera free status, which ultimately provides for the total elimination of vaccination.

Your Committee is pleased to note that as recommended in its 1962 Report, a standard procedure guide has been developed to aid in the field diagnosis of hog cholera. While it is recognized that there are still areas in which this guide needs improvement, the Animal Disease Eradication Division, cooperating with the various states engaged in the program, has obtained supplemental information and will continue with the evaluation of this guide in field use.

The Secretary of Agriculture's Hog Cholera Advisory Committee was this year again invited and participated in the deliberations of our committee. We feel that the exchange of ideas between members of both committees has been of great value to the swine industry in evaluating and developing the nationwide program for the eradication of this disease.
EXAMINATION OF BOVINE SPECIMENS SUSPECTED OF BEING TUBERCULOUS

H. A. McDaniel, D.V.M.; D. G. Howell, B.S., D.V.M.;
K. C. Sherman, B.S., D.V.M.; D. R. Cassidy, D.V.M., M.S.

Ames, Iowa

Disease eradication and control programs depend heavily upon diagnostic procedures. Such procedures not only serve for detection of infection on an individual and herd basis, but cumulative data obtained from large numbers of post mortem and laboratory examinations provide us with a means of constant re-evaluation of diagnostic tools. A major effort of the Pathology Section, Diagnostic Services, National Animal Disease Laboratory, has been the histopathologic examination of tuberculous-like lesions from both tuberculosis reactor and routinely slaughtered cattle. This effort has provided a more definitive diagnosis of the disease as well as a means of gathering information that should be valuable to the tuberculous eradication program.

The histopathologic confirmation of tuberculosis is a key factor in locating infected herds throughout the country. Follow-up tuberculin testing of herds based upon the detection of suspicious lesions in non-tested cattle is a valuable adjunct to the tuberculosis eradication program. Davis and Anderson\(^1\) stated that nearly 15 percent of all tuberculin reactors were uncovered annually in this manner. Ranney cited a marked contrast of 33 cattle tested to find one reactor in trace back operations as compared to 919 cattle tested to find one reactor under other program tests during 1962.\(^2\) Although only 0.34 percent of the total cattle tested for tuberculosis in 1962 were tested as a result of traceback on lesions disclosed at slaughter, follow-up tuberculin testing disclosed 8.6 percent of all reactor animals and 22.5 percent of all reactors with lesions.\(^2\)

The effectiveness of this procedure is apparent, but the possibility of error in reliance on gross lesions only for a diagnosis of tuberculosis should be emphasized. Davis and Anderson reported that 41 (53.3 percent) of 77 suspected tuberculous lesions found in non-tested cattle were granulomas due to other causes.\(^3\) Subsequently, laboratory examination of an additional 418 cases by these workers showed that 45.7 percent of the lesions suspected of being tuberculous on gross examination were not tuberculous.\(^1\)

Efforts of this section also have included histopathologic examination of specimens from reactor cattle with or without gross lesions of

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Our appreciation is extended to Drs. E. M. Ellis, Discipline Leader and W. D. Yoder, Project Leader, Bacteriology Section, Diagnostic Services, NADL, for the bacteriological findings reported in this study.

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EXAMINATION OF BOVINE T.B. SPECIMENS

A number of skin lesion cases have been examined. An increasing number of "divided specimens," i.e., specimens preserved in 10 percent formalin for the pathology section and in borax or hypochlorite solution for the bacteriology section, are being submitted. This allows comparison of findings from both histopathologic and bacteriologic examination of the same lesion or, at least, lesions taken from the same general area.

The purpose of this paper is to report the results of these examinations and to discuss the relationship of these findings to the program for the eradication of bovine tuberculosis. The cases reported herein were received from the Meat Inspection and Animal Disease Eradication Divisions of the Agricultural Research Service, U.S. Department of Agriculture, as well as state and municipally inspected abattoirs.

RESULTS

The results of the histopathologic examination of 1,608 bovine specimens suspected of being tuberculous on gross examination are shown in Table I. Eight hundred and one (49.8 percent) of the total 1,608 specimens thought to be tuberculous on gross examination were confirmed as tuberculous on histopathologic examination. An additional 138 (8.6 percent) were

| TABLE I |
|-----------------|-----------------|-----------------|
| **Histopathologic Evaluation of 1,608 Bovine Specimens Suspected of Being Tuberculous on Gross Examination** | **Specimens from Reactor Cattle** | **Specimens from Routinely Slaughtered Cattle** |
| **Histopathologic Findings** | **Tuberculosis** | **Suggestive** | **Actinobacillosis** | **Actinomycosis** | **Coccidiodomycosis** | **Mucormycosis** | **Other Mycotic** | **Abscess** | **Neoplasm** | **Parasitic** | **Miscellaneous** | **No Microscopic Lesion** | **Total** |
| | Confirmed* | | | | | | | | | | | | | | |
| | 489 | | | | | | | | | | | | | | 801 |
| Tuberculosis | | | | | | | | | | | | | | |
| Suggestive** | 67 | | 71 | | | | | | | | | | | | 138 |
| Actinobacillosis | 33 | | 75 | | | | | | | | | | | | 108 |
| Actinomycosis | 7 | | 13 | | | | | | | | | | | | 20 |
| Coccidiodomycosis | 2 | | 24 | | | | | | | | | | | | 26 |
| Mucormycosis | 1 | | 12 | | | | | | | | | | | | 13 |
| Other Mycotic | 2 | | 5 | | | | | | | | | | | | 7 |
| Abscess | 33 | | 68 | | | | | | | | | | | | 101 |
| Neoplasm | 8 | | 76 | | | | | | | | | | | | 84 |
| Parasitic | 17 | | 24 | | | | | | | | | | | | 41 |
| Miscellaneous | 55 | | 104 | | | | | | | | | | | | 159 |
| No Microscopic Lesion | 74 | | 36 | | | | | | | | | | | | 110 |
| **Total** | 788 | | 820 | | | | | | | | | | | | 1,608 |

*Tuberculous lesions in which acid-fast organisms were demonstrated.

**Lesions resembling tuberculosis in which neither acid-fast organisms nor other etiologic agents were demonstrated.

†For the purpose of this paper, these are considered as non-tested animals.
considered suggestive of tuberculosis even though acid-fast bacilli were not seen. A diagnosis of tuberculosis was confirmed by histopathologic examination in 489 (62 percent) of the 788 specimens submitted from reactor animals. An additional 67 (8.5 percent) were considered suggestive of tuberculosis upon histopathologic examination. In the case of the routinely slaughtered animals, 312 (38 percent) of 820 specimens submitted were verified as tuberculous upon histopathologic examination and 71 (8.6 percent) were considered suggestive.

Based on the data from Table I at least 29.2 percent of the lesions submitted from reactor animals and 53.4 percent of those from routinely slaughtered animals probably were due to causes other than tuberculosis. Actinobacillosis was the one condition most often confused with tuberculosis upon gross examination in both reactor and regular kill animals. Abscesses and neoplasms also were often diagnosed in specimens suspected of being tuberculous upon gross examination.

Three hundred and twenty specimens were submitted for histopathologic examination from reactor animals with no gross lesions of tuberculosis. Granulomatous lesions containing acid-fast bacilli were found in seven (2.2 percent of these specimens and lesions suggestive of tuberculosis were found in an additional 25 (7.8 percent) of the cases.

The results of the histopathologic examination of 104 skin lesions are shown in Table II. Lesions typical of a tuberculoid dermatitis were observed in 97 of the 104 skin specimens and acid-fast bacilli were demonstrated in 78 (80 percent) of this group. Fifty-seven of these 97 lesions (58.7 percent) were from reactor animals with no other gross lesions of tuberculosis.

Two hundred and forty-four specimens from which acid-fast organisms had been cultured were examined histopathologically. The specimens were submitted from both reactor and routinely slaughtered animals. The frequency with which granulomatous tissue responses were noted in these specimens is shown in Table III. Eighty-eight (36.1 percent) of the 244 isolates were *Mycobacterium bovis*, 61 (25 percent) were Runyon Group III

---

**TABLE II**

<table>
<thead>
<tr>
<th>Histopathologic Findings</th>
<th>Reactor Cattle</th>
<th>Routinely Slaughtered Cattle</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulomas with acid-fast organisms</td>
<td>45</td>
<td>33</td>
<td>78</td>
</tr>
<tr>
<td>Granulomas (No agent demonstrated)</td>
<td>12</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Parasitic</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Abscess</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Actinobacillosis</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neoplastic</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58</strong></td>
<td><strong>46</strong></td>
<td><strong>104</strong></td>
</tr>
</tbody>
</table>
### TABLE III

Types of Acid-fast Organisms Cultured and Occurrence of Granulomatous Lesions in 244 Suspected Tuberculosis Specimens from the Bovine*

<table>
<thead>
<tr>
<th>Organism Recovered</th>
<th>Number of Specimens in which Acid-fast Bacilli were Demonstrated Histopathologically</th>
<th>Number of Isolates</th>
<th>Specimens Containing Granulomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor</td>
<td></td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Routinely Slaughtered Cattle</td>
<td></td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>Reactor</td>
<td></td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>Routinely Slaughtered Cattle</td>
<td></td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>61</td>
<td>50</td>
</tr>
<tr>
<td>Reactor</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Routinely Slaughtered Cattle</td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
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<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Reactor</td>
<td></td>
<td>46</td>
<td>30</td>
</tr>
<tr>
<td>Routinely Slaughtered Cattle</td>
<td></td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>88</td>
<td>56</td>
</tr>
</tbody>
</table>

*These bacteriologic findings were obtained from the records of the Bacteriology Section, Diagnostic Services.

**Biochemical tests fail to differentiate these organisms. Animal inoculation studies are underway to obtain more definitive identification.

Mycobacteria or _M. avium_, 88 (36.1 percent) were Runyon Group IV mycobacteria, and seven (2.8 percent) were Runyon Group II mycobacteria. _M. bovis_ constituted 43 percent of the isolates from reactor animals. The Runyon Group III mycobacteria or _M. avium_ made up 25.2 percent of these isolates, the Runyon Group IV mycobacteria made up an additional 30.5 percent and the Runyon Group II mycobacteria constituted the remaining 1.3 percent. _M. bovis_ and Runyon Group III mycobacteria or _M. avium_ each constituted 24.7 percent of the isolates from the routinely slaughtered animals. The Runyon Group IV mycobacteria constituted 45.2 percent of the isolates and the Runyon Group II mycobacteria comprised the remaining 5.4 percent.
Granulomas were demonstrated on histopathologic examination in 93.2 percent of the specimens from which *M. bovis* was recovered. Acid-fast organisms were demonstrated in 90.2 percent of these lesions. In those specimens from which Runyon Group III mycobacteria or *M. avium* organisms were isolated, granulomas were demonstrated in 82 percent of the cases and acid-fast bacilli were seen in 88 percent of these granulomas. Granulomas were observed in six of the seven specimens from which the Runyon Group II mycobacteria were isolated. Four of these granulomas contained acid-fast bacilli. Granulomatous lesions were observed in 64 percent of the tissue from which the Runyon Group IV mycobacteria were recovered. Acid-fast bacilli were seen in 66 percent of these lesions.

Histopathologic and/or bacteriologic examination of bovine specimens suspected of being tuberculous revealed that other conditions or organisms were found in 27 (30.7 percent) of the 88 specimens from which the Runyon Group IV mycobacteria were recovered. These are shown in Table IV.

**DISCUSSION**

The value of the histopathologic examination of lesions resembling tuberculosis that are detected on postmortem examination has been re-emphasized in this paper. It is apparent that histopathologic examination of such lesions could reduce by approximately 50 percent the number of animals that would have to be traced by state and federal regulatory officials if sole dependence was placed on postmortem lesions at autopsy. This figure is strikingly similar to one reported by Davis and Anderson in a report of 108 cases suspected of being tuberculous on gross postmortem examination. They stated that 48.2 percent of the lesions submitted as suspected tuberculosis were found to be other conditions upon laboratory

**TABLE IV**

| Conditions Observed and/or Organisms Cultured From Specimens of Bovine Origin From Which Runyon Group IV mycobacteria Were Recovered* |
|---------------------------------------------------------------|-------|
| Actinomycosis                                                 | 1     |
| Actinobacillosis                                              | 7     |
| Abscesses                                                     | 5     |
| Mucormycosis                                                  | 1     |
| Neoplasm                                                      | 3     |
| Parasites                                                     | 3     |
| Runyon Group II mycobacteria                                  | 1     |
| Runyon Group III mycobacteria *M. avium*                      | 2     |
| *M. bovis*                                                    | 1     |
| *M. fortuitum*                                                | 3     |
| **Total**                                                     | 27    |

*Bacteriologic findings were obtained from the records of the Bacteriology Section, Diagnostic Services.
examination. We would re-emphasize the statement of Davis and Anderson that this by no means casts reflection on the judgment of veterinary inspectors. Quite to the contrary, since many of these cases actually are indistinguishable from tuberculous lesions grossly, it reflects sound judgment that such specimens be forwarded to a laboratory for a more definitive diagnosis.

It would seem that histopathologic confirmation of suspected tuberculous lesions is worthwhile even when such lesions are taken from reactor animals. The data in Table I suggests that 29 percent of the gross lesions in reactor animals probably were due to other causes.

An extensive discussion of the no gross lesion (NGL) problem is beyond the scope of this paper. However, it seems worthy of note that lesions resembling tuberculosis were observed in 32 (10 percent) of the 320 specimens submitted from reactor cattle with no gross lesions of tuberculosis. Microscopic lesions of tuberculosis with acid-fast bacilli were seen in seven (2.2 percent) of the 320 specimens and an additional 25 (7.8 percent) had lesions suggestive of tuberculosis although acid-fast bacilli were not seen. Crawford indicated more than 25 years ago that one of the factors explaining the phenomenon of an allergic response to tuberculin in the absence of visible glandular or visceral lesions might well be due to the presence of tuberculous lesions which are either occult or too minute to be observed macroscopically.4 Mallmann has reported microscopic lesions characteristic of tuberculosis in tissues from NGL reactors.5 The "NGL" terminology is based only on prima facie evidence indicating lesions were not observed grossly under conditions that are not conducive to a thorough search of the carcass for minute lesions. Although the occurrence of such lesions may not be predominant among the reasons explaining tuberculin response in the absence of gross lesions, their consideration as another contributory factor must not be overlooked.

The data in Table II relative to the histopathologic examination of skin lesions is in essential agreement with the statement of Traum that it is generally accepted that cattle with skin lesions containing acid-fast bacilli will show tuberculin sensitivity to the intradermal test in approximately 50 percent of the cases.6 It was shown in Table II that 45 (58 percent) of the 78 skin lesions in which tuberculoid lesions and acid-fast bacilli were demonstrated were from reactors showing no other gross lesions of tuberculosis. Acid-fast saprophytes and other mycobacteria have been isolated from tuberculoid skin lesions, but their role in the pathogenesis of the lesions is not clear.5,6 Although the disease significance of such lesions is minimal, their association with tuberculin allergy makes them of more than passing concern to all interested in the control and eradication of tuberculosis.

The data in Table III indicated that mycobacteria other than M. bovis constituted approximately two-thirds of the total number of isolates from bovine specimens. They made up 57 percent of the isolates from reactor animals and approximately 75 percent of those from routinely slaughtered animals. Crawford mentioned that other acid-fast organisms are capable of setting up a state of sensitization to tuberculin in cattle
without producing apparent lesions. The observation that granulomatous tissue responses were observed in approximately two-thirds of the field specimens from which acid-fast bacilli other than *M. bovis* were recovered seems of interest in this regard. Numerous reports in the literature have discussed *M. avium* infection in cattle and its association with production of lesions and/or sensitization to tuberculin. Research is under way at Michigan State University relative to the pathogenicity of a selected group of atypical mycobacteria for calves. The role of acid-fast organisms other than *M. bovis* in the pathogenesis of the lesions observed in our field cases is not clear. It must be borne in mind that the pathologist could have observed lesions unrelated to the acid-fast organisms isolated by the bacteriologist, since different sections of the tissue were examined by each group. It may also be that the so-called atypical mycobacteria became established in old lesions originally due to *M. bovis*. The possibility that this occurs with atypical mycobacteria in old lesions originally caused by *M. tuberculosis* in the human has been advanced by Wolinsky, et al. A third consideration can be drawn from the data in Table IV where it was shown that the Runyon Group IV mycobacteria were found in association with other mycobacteria as well as with granuloma-producing agents other than mycobacteria. These data suggest that the organisms might become established in a variety of conditions where tissue damage occurs, i.e., parasitism, neoplasms, mycotic conditions, abscesses and tuberculosis.

Such factors should caution against ascribing to great significance to these organisms in the pathogenesis of the lesions observed. On the other hand, other factors would indicate that a cause and effect relationship between the organisms recovered and the lesions observed cannot be overlooked. Individual lesions often were divided for examination by each section or were, at least, taken from the same general area. This would lessen the possibility that lesions observed by the pathologist had no relation to organisms recovered by the bacteriologist. Although it seems a good possibility that some of the atypical mycobacteria would become established in old *M. bovis* lesions, the appearance of some of the lesions indicated an active process rather than an old lesion. It would seem that if granuloma producing agents other than the acid-fast organisms were responsible for the lesions they would be demonstrated with greater frequency upon histopathologic examination. These factors would suggest that further consideration be given to the influence of acid-fast organisms other than *M. bovis* in the production of both an allergic response to tuberculin and a cellular response in the tissues of the bovine.

**SUMMARY**

1. Histopathologic examination of 1,608 bovine specimens suspected of being tuberculous on gross examination resulted in confirmation of the clinical or postmortem diagnosis of tuberculosis in 49.8 percent of all cases; twenty-nine percent of the lesions from reactor animals and 53 percent of those from routinely slaughtered animals probably were due to causes other than tuberculosis.
2. Actinobacillosis, abscesses and neoplasms were conditions most often confused with tuberculosis upon gross examination.

3. Confirmatory or suggestive histopathologic lesions of tuberculosis were disclosed in 32 (10 percent) of 320 specimens submitted from reactor cattle in which no gross lesions were observed.

4. Lesions of a tuberculoid dermatitis were observed in 97 of 104 skin specimens and acid-fast bacilli were demonstrated in 78 of the cases. Forty-five (58 percent) of these 78 cases were from reactor animals with no other gross lesions of tuberculosis.

5. Eighty-eight (36.1 percent) of a total of 244 acid-fast isolates from suspected tuberculosis cases were *M. bovis*, 61 (25 percent) were either Runyon Group III mycobacteria or *M. avium*, 88 (36.1 percent) were Runyon Group IV mycobacteria and seven (2.8 percent) were Runyon Group II mycobacteria.

6. Granulomatous lesions were demonstrated in 93.2 percent of the specimens from which *M. bovis* was cultured and acid-fast bacilli were demonstrated in 90.2 percent of these lesions. In the case of Runyon Group III mycobacteria or *M. avium*, granulomas were demonstrated in 82 percent of the tissues and acid-fast bacilli were observed in 88 percent of these lesions; granulomatous lesions were observed in 64 percent of the tissue from which the Runyon Group IV mycobacteria were recovered. Acid-fast bacilli were seen in 66 percent of these lesions.

7. Concurrent conditions, i.e., mycoses, neoplasms, parasitisms, abscesses, and other acid-fast organisms were disclosed in 27 of the 88 cases from which the Runyon Group IV mycobacteria were cultured.

8. The relationship of these findings to the program for the eradication of bovine tuberculosis is discussed.

REFERENCES


INVESTIGATIONS OF THE ROLE OF HEAT KILLED MYCOBACTERIA AND FEED SUPPLEMENTS OF ANIMAL ORIGIN IN PRODUCING TUBERCULIN HYPERSENSITIVITY IN CATTLE


East Lansing, Michigan

INTRODUCTION

The total number of tuberculin reactor cattle slaughtered in the United States in the fiscal year ending June 30, 1963 was 82752 which was a considerable economic loss. Seventy-five percent of the reactors slaughtered had no gross lesions of tuberculosis. High percentages of no gross lesion (NGL) reactors have been found during the last several years. Microscopic granulomata have been found in the tissues of NGL reactors, and Mycobacterium bovis and atypical mycobacteria have been isolated repeatedly by workers of the Tuberculosis Project of Michigan State University.\(^4\) Factors other than mycobacterial infections have been suggested as causes of tuberculin hypersensitivity. One of these factors is the ingestion of killed or living acid-fast organisms that may be present in the ration, particularly in meat and bone scrap and steamed bone meal.

A limited study conducted by Whitehead and Corson\(^6\) indicated that guinea pigs fed killed tubercle bacilli added to their normal ration for 13 to 15 days were sensitive to tuberculin. Control guinea pigs fed only the normal ration for the same period of time were not sensitive to tuberculin. A subsequent study conducted by Stockton and Newman\(^5\) in which one group of guinea pigs were fed up to one billion cells of heat-killed \(M.\) bovis and another group were fed up to one billion cells of heat-killed \(M.\) avium daily for 28 days did not substantiate these findings.

In additional preliminary studies acid-fast organisms were found to be present in large numbers in samples of meat and bone scrap and steamed bone meal obtained from various retail sources. The organisms were observed microscopically in a smear of the supplement stained by the Ziehl-Neelsen technique.

*From the Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan.

**From the Animal Disease Eradication Division, Agricultural Research Service, U. S. Department of Agriculture, Lansing, Michigan.

This study was supported by Research and Marketing Act Contract number 12-14-100-5786 (45) and Memorandum of Understanding, Line Project No. ADP al-13 from the Animal Disease and Parasite Research Division and the Animal Disease Eradication Division of the Agricultural Research Service, U. S. Department of Agriculture, respectively.
Because of the importance to the livestock industry of the possibility of feed supplements of animal origin causing tuberculin hypersensitivity, and because high numbers of acid-fast organisms were found to be present in such supplements, a study using cattle as the test animal was conducted. In addition to feeding the supplements of animal origin, killed mycobacteria in known numbers were fed.

The purpose of this study was to determine if heat-killed Mycobacterium avium, heat-killed M. bovis, meat and bone scrap, or steamed bone meal fed to cattle, caused the animals to react to the intradermal tuberculin tests now used in the tuberculosis eradication program.

MATERIALS AND METHODS

Experimental Animals. Sixty nonpregnant predominantly Guernsey crossbred heifers 15 to 24 months old were obtained from a herd in which all animals had been tuberculin negative for a long period of time. Immediately prior to purchase all animals in the herd were negative to the caudal fold tuberculin test. The animals purchased for this study had no detectable response when retested in the cervical region with 0.1 cc mammalian tuberculin,* 0.1 cc avian tuberculin** and 0.2 cc johnin.*** Precautions were taken during the transportation of the animals to prevent contamination with microorganisms from other livestock. Tests were negative for internal parasites, antibodies for Leptospira pomona and antibodies for Brucella abortus.

Housing of the Animals. The barn in which the experiment was conducted had not housed animals during the past five years. It was divided into four units and equipped so that 15 animals were stanchioned in each unit. The units and mow area used to store feed and bedding were cleaned and disinfected. The walls, ceilings and stanchions of each unit were painted. Each unit was screened to exclude flies and the mow area to exclude birds. The routes for removal of manure from each unit did not cross that of another. Air was exhausted to the exterior from each unit.

Experimental Ration. Good quality baled alfalfa hay and water were given the animals of all units. Each animal was fed approximately four pounds of a mixed grain ration per day. The two grain rations fed were formulated as follows: 1) Control Ration. 1,777 lb. No. 2 yellow shelled corn, 200 lb. 50 percent solvent process soybean oil meal, 23 lb. dicalcium phosphate, 20 lb. trace mineralized salt. 2) Animal Origin Supplement Ration. 1,780 lb. No. 2 yellow shelled corn, 200 lb. meat and bone scrap (50 percent protein), 20 lb. steamed bone meal, 20 lb. trace mineralized salt, 100 lb. molasses. The meat and bone scrap and the steamed bone meal were obtained from various representative sources. The animals in

* Tuberculin, mammalian, intradermic produced for the Agricultural Research Service, U. S. Department of Agriculture.
** Tuberculin, avian, intradermic, produced for the Agricultural Research Service, U. S. Department of Agriculture.
*** Johnin, intradermic, produced for the Agricultural Research Service, U. S. Department of Agriculture.
Unit A were fed the control ration. The control ration to which heat-killed \textit{M. bovis} was added was fed to the animals in Unit B and the Control ration to which heat-killed \textit{M. avium} was added was fed to the animals in Unit C. Animals in Unit D were fed the Animal Origin Supplement Ration. The \textit{M. bovis} and \textit{M. avium} additives were prepared and fed as follows: Living organisms grown in Dubos—one percent dextrose broth were mixed with ground beef, heat-killed (121°C moist heat for 30 minutes) and mixed in a sterile blender for three to five minutes. The resulting slurry was then mixed with sterile saline containing 0.5 percent molasses and the mixture dispensed aseptically into sterile screw-capped tubes. The dilutions were made so that each tube contained approximately \(5 \times 10^{8-9}\) organisms. Each animal in Units B and C was fed the contents of one tube daily by adding it to the control ration.

Tuberculin tests were performed according to U. S. Department of Agriculture directions by experienced A.D.E. personnel (C. A. Niles). Alternate sides of the animals were used as test sites for successive tests. Tests were made with 0.1 cc mammalian tuberculin. All animals were tested in the caudal fold 60 days after they were received and 42 days before the feeding of the experimental rations was started. After the feeding of the experimental rations had begun the 15 animals in each unit were divided into three lots of five animals each. Caudal fold tests were performed on the different lots of five animals from each unit at 20, 30 and 40 days after the start of feeding the experimental rations. All animals were again tested using the caudal fold test 100 days, and in the cervical region 160 days after the start of feeding the experimental rations. Skin thicknesses at the injection sites were measured before injections were made. All detectable responses were measured and the increase in skin thickness recorded. The tests were read at two, 24, 48 and 72 hours following injection. All skin thickness measurements were made with a Haputner dermal thickness gage. To determine the variation in normal skin thicknesses all injection sites were measured on three consecutive days one week prior to the 160 day tests.

\textbf{RESULTS AND DISCUSSION}

The maximum difference obtained when skin thicknesses of the test sites were measured before injection of tuberculin was 1.9 mm at the cervical site, 1.4 mm at the left caudal fold site and 1.2 mm at the right caudal fold site.

Tables I, II and III were compiled from measurements made at the official time of reading the tests (48 hours cervical; 72 hours caudal fold). Indicated therein are the numbers of animals being fed each ration and having the following: 1) Any detectable response (Table I), 2) greater than two mm response (Table II) or 3) an \(X^2\) (increase to two times normal skin thickness) or greater response (Table III).

One animal fed the \textit{M. avium} ration and one fed the animal origin ration had detectable caudal fold responses (Table I). Three animals being fed the \textit{M. avium} ration and two animals fed the animal origin ration had a
TABLE I
Number of Animals with Any Detectable Response at the Official Reading Times (48 hr. Cervical; 72 hr. Caudal Fold)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Caudal Fold Tests</th>
<th>Cervical Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animal Origin</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE II
Number of Animals with Response Greater than Two mm at the Official Reading Times (48 hr. Cervical; 72 hr. Caudal Fold)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Caudal Fold Tests</th>
<th>Cervical Tests</th>
</tr>
</thead>
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<tr>
<td>Control</td>
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<td>0</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
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<td>0</td>
</tr>
<tr>
<td><em>M. avium</em></td>
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</tr>
<tr>
<td>Animal Origin</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE III
Number of Animals with an X2 or Greater Response at the Official Reading Time (48 hr. Cervical; 72 hr. Caudal Fold)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Caudal Fold Tests</th>
<th>Cervical Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
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</tr>
<tr>
<td><em>M. bovis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animal Origin</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

detectable response when tested in the cervical region. The animals responding to the caudal fold test did not respond to the cervical test and vice versa. It is emphasized that the responses indicated in this table are any that were palpable and may have been as slight a difference as 0.1 mm which is not detectable to the naked eye.

The two caudal fold responses noted in Table I measured 2.1 mm each. When one of these responses was compared to the thickness of the opposite caudal fold there was only a 0.5 mm difference found. A 2.1 mm response is very small. If these animals had been classified as suspects initially, they would have been classified as negative on subsequent tests.

No animals responded with an X2 or greater swelling (Table III). It is suggested in the instructions from the United States Department of
### TABLE IV
Number of Animals with Any Responses to 0.1 cc Mammalian Tuberculin at Various Reading Times Over the Number of Animals Tested

<table>
<thead>
<tr>
<th>Ration</th>
<th>Days Fed Ration</th>
<th>2 Hours</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>20 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>30 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>40 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>100 days</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>160 days</td>
<td>(cervical)</td>
<td>15/15</td>
<td>4/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>M. bovis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>20 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>30 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>3/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>40 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>100 days</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>7/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>160 days</td>
<td>(cervical)</td>
<td>15/15</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>M. avium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>20 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>2/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td>30 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>40 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>100 days</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>5/15</td>
<td>1/15</td>
<td>1/15</td>
</tr>
<tr>
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<td>(cervical)</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>(caudal fold)</td>
<td>15/15</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>20 days</td>
<td>(caudal fold)</td>
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<td>0/5</td>
</tr>
<tr>
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<td>(caudal fold)</td>
<td>5/5</td>
<td>1/5</td>
<td>1/5</td>
<td>1/5</td>
</tr>
<tr>
<td>40 days</td>
<td>(caudal fold)</td>
<td>5/5</td>
<td>2/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td>100 days</td>
<td>(caudal fold)</td>
<td>15/15</td>
<td>5/15</td>
<td>1/15</td>
<td>0/15</td>
</tr>
<tr>
<td>160 days</td>
<td>(cervical)</td>
<td>15/15</td>
<td>5/15</td>
<td>2/15</td>
<td>1/15</td>
</tr>
</tbody>
</table>
# Table V

Number of Animals with Greater than 2 mm Response to 0.1 cc Mammalian Tuberculin at Various Reading Times Over the Number of Animals Tested

<table>
<thead>
<tr>
<th>Ration</th>
<th>Days Fed Ration</th>
<th>2 Hours</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior (caudal fold)</td>
<td></td>
<td>10/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>20 days (caudal fold)</td>
<td></td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>30 days (caudal fold)</td>
<td></td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
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<td></td>
<td>5/5</td>
<td>0/5</td>
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<td>0/5</td>
</tr>
<tr>
<td>100 days (caudal fold)</td>
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<td>0/15</td>
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<td>0/15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior (caudal fold)</td>
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<td>11/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
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<tr>
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<tr>
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<td>0/5</td>
</tr>
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<td>0/5</td>
</tr>
<tr>
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<tr>
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<td>12/15</td>
<td>3/15</td>
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<td>0/15</td>
</tr>
<tr>
<td><strong>M. avium</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior (caudal fold)</td>
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<td>0/15</td>
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<td>0/5</td>
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<td>0/5</td>
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<tr>
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<td>0/5</td>
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</tr>
<tr>
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<td>1/15</td>
<td>1/15</td>
<td>1/15</td>
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<tr>
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<td></td>
</tr>
<tr>
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<td>0/15</td>
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<tr>
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<td>0/5</td>
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</tr>
<tr>
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<td>0/5</td>
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<td>0/5</td>
</tr>
<tr>
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<td>10/15</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
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<td></td>
<td>14/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
</tbody>
</table>
TABLE VI

Number of Animals with X2 or P1 (3 mm) or Greater Response to 0.1 cc Mammalian Tuberculin at Various Reading Times Over the Number of Animals Tested

<table>
<thead>
<tr>
<th>Ration</th>
<th>Days Fed Ration</th>
<th>2 Hours</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>prior (caudal fold)</td>
<td>4/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>20 days (caudal fold)</td>
<td>3/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>30 days (caudal fold)</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>40 days (caudal fold)</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>100 days (caudal fold)</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>160 days (cervical)</td>
<td>2/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>M. bovis</td>
<td>prior (caudal fold)</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>20 days (caudal fold)</td>
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<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>30 days (caudal fold)</td>
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<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>40 days (caudal fold)</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>100 days (caudal fold)</td>
<td>4/15</td>
<td>0/15</td>
<td>0/15</td>
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</tr>
<tr>
<td></td>
<td>160 days (cervical)</td>
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<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>M. avium</td>
<td>prior (caudal fold)</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>20 days (caudal fold)</td>
<td>3/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>30 days (caudal fold)</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>40 days (caudal fold)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>100 days (caudal fold)</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>160 days (cervical)</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
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<td>0/15</td>
</tr>
<tr>
<td></td>
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<td>0/5</td>
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<tr>
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<td>30 days (caudal fold)</td>
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<td></td>
<td>160 days (cervical)</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
</tbody>
</table>
Agriculture\textsuperscript{1} that an animal with an X2 or greater response usually be classified as a reactor.

The number of animals that responded at various reading times with any response, greater than two mm response, or an X2 or P1 (three mm) response or greater are indicated in Tables IV, V and VI respectively. A detectable response to mammalian tuberculin was found at two hours in every animal each time it was tested including the test conducted prior to start of feeding the experimental rations. Seventy-nine percent of these responses were greater than two mm and 23 percent were X2 or P1 or greater responses. The immediate responses could usually be observed as early as 10 to 15 minutes following injection. In all cases these immediate responses had decreased in size by the official reading time.

At the 24 hour reading 24 percent of the animals had a detectable response, three percent had responses that were greater than two mm and none had X2 or P1 or greater response. At 48 hours four percent had a detectable response, 0.8 percent had greater than two mm and none of the animals had an X2 or P1 or greater response. At 72 hours following the injection of tuberculin, only one percent had any detectable response, 0.8 percent a response greater than two mm and none an X2 or P1 or greater response.

The cause of the immediate responses is not known. While they did seem to increase in duration as the study progressed, they were not considered to be caused by the experimental rations as they were also noted on all animals in the control group.

**SUMMARY**

Sixty nonpregnant predominantly Guernsey crossbred heifers were fed one of four different rations for 160 days to determine if any of the rations would induce delayed hypersensitivity in the animals as detected with 0.1 cc mammalian tuberculin injected intradermally. The animals were obtained from a herd with no tuberculin reactors and had no detectable response to mammalian or avian tuberculins or johnin when tested in the caudal fold and cervical regions. They were maintained during the study in four isolated groups of 15 each. The control group was fed a ration in which the protein concentrate was soybean oil meal and the mineral concentrate was dicalcium phosphate. The second and third groups were fed the control ration to which daily was added $5 \times 10^8$ heat-killed (121°C moist heat for 30 minutes) *Mycobacterium bovis* and *Mycobacterium avium* respectively. The fourth group was fed a ration in which the protein concentrate was meat and bone scrap and the mineral concentrate steamed bone meal. Tuberculin tests using 0.1 cc mammalian tuberculin were performed on all animals at three different times. Some were tested at 20, 30 or 40 days, and all were tested at 100 and 160 days following the start of feeding the experimental rations. No animal was classified as a reactor at the official reading time.
ACKNOWLEDGEMENTS

The authors wish to thank the members of the Animal Disease Eradication Division and the Animal Disease and Parasite Research Division of the Agricultural Research Service, U. S. Department of Agriculture, the Michigan Department of Agriculture, the Michigan Department of Mental Health and Michigan State University who cooperated in the design and execution of this study.

REFERENCES

THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

A. F. Ranney*

Hyattsville, Maryland

The need for continued vigilance in our tuberculosis eradication program was never more obvious than it is today. While the incidence of bovine tuberculosis is relatively low, we must remember that tuberculosis is still dispersed country-wide. This is illustrated in Figure 1.

In a discussion of problems to be solved in eradicating tuberculosis from humans, Dr. Dred L. Soper,1 a strong proponent of disease eradication as an absolute goal, makes the following comments:

"The acceptance of the concept of eradication in place of control (reduction) forces a radical psychological and administrative change in attitude toward the existence of low-incidence tuberculosis in the community. This change is based on the fundamentally different objectives of control and of eradication.

*Dr. A. F. Ranney, Acting Chief Staff Officer, Tuberculosis Eradication, Animal Disease Eradication Division, Agricultural Research Service, Hyattsville, Maryland.

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"The objective of control is to reduce the incidence of a given disease to a low level and to maintain this low level forever.

"The objective of eradication is completely to eliminate the possibility of the occurrence of a given disease, even in the absence of all preventive measures. This definition, modified by the phrase 'unless reintroduction occurs,' applies also to local area, state, national, and regional eradication. . . .

"In control, one tends to lose interest in a disease at the point where, in eradication, many times the greatest difficulties are encountered. . . .

"Eradication has no meaning except as an absolute."

How do these statements coincide with our philosophy and efforts to eradicate tuberculosis from domestic livestock? First, we can congratulate ourselves on the fact that eradication of tuberculosis has always been and still is our goal. Second, for several years after reaching our intermediate goal of a national modified accredited area status for bovine tuberculosis interest in and attention to tuberculosis eradication tended to decline as we maintained little more than a control program. The problems encountered during this period are not unlike those frequently encountered in other disease elimination programs as the incidence of the disease decreases. Third, the continued interest in bovine tuberculosis eradication in recent years is exemplified by the fact that the Committee on Tuberculosis of this Association is sincerely striving to set up criteria for areas that may be classed as tuberculosis free.

We are generally recognizing the fact that conditions change as we progress toward eradication, and again I quote from Soper:1 "Actual experience does not indicate that the factors which effect the gross reduction of an infection will be necessarily effective at the lowest levels of infection."

The number of cattle tested during the past year was approximately a million less than for the fiscal year 1962, 8.3 million as compared to 9.2 million. This is the smallest number reported under test during an annual period since 1959. It is interesting to note that the percent classed as reactors declined during the past five years from 0.23 to 0.10. This reduction in rate of infection is especially significant when it is considered that the number of reactors with extensive tuberculous lesions rose 18.6 percent while the carcasses reported with similar lesions on regular-kill meat inspection declined 18.7 percent during the same period (1959-1963). Emphasis on professional judgment when interpreting tuberculin tests along with the use of the suspect classification are factors to be considered in this reduction in percentage of reactors reported.

Meat Inspection data continue to show a decline in the number of carcasses retained for tuberculosis as well as those condemned or passed for cooking.

The importance of concentrating our efforts on herds where infection is most likely to exist is in line with the program priorities established by our Committee on Tuberculosis in 1961.2 The bulk of the reactors with
generalized tuberculous lesions were found as a result of testing herds after tracing the origin of cattle disclosing lesions on regular-kill (ADE 6-35), and intensive work on retests of known infected herds.

Intensive epidemiological studies are revealing some very interesting and valuable information. One particularly interesting case involves a State Institutional herd in Virginia from which reactors to the tuberculin test had been disclosed as a result of repeated tests covering a period of several years. Selected tissues from no-gross-lesion reactors examined at the National Animal Disease Laboratory (NADL) disclosed *Mycobacterium avium*. Tissues from pigeons captured on these premises also examined at NADL resulted in the isolation of *Mycobacterium avium* or Runyon Group III mycobacteria. This points up the need for diligent investigations to locate hidden foci of infection. As we reduce the amount of bovine type tuberculosis other types of tuberculosis become more significant in our eradication efforts.

A practitioner in Montana performing a post-mortem on a purebred bull found a single calcified mesenteric lesion. Examination of lesions at the State diagnostic laboratory revealed evidence of tuberculosis. As a result of this report the remaining cattle on the premises were tested. A purebred bull responded. A tuberculous lesion in a mesenteric lymph node was found following slaughter of this animal. On laboratory examination of this lesion *M. avium* was identified. Thirty-five old hens, some of which revealed advanced lesions of tuberculosis had access to the bull quarters on the ranch. The flock of hens was sacrificed and destroyed by incineration on the premises and the premises cleaned and disinfected. On retest of the cattle at a later date all animals were reported negative.

A herd of 47 animals tested for sale in Wisconsin revealed 38 reactors, 10 of which were found to have macroscopic lesions in mesenteric lymph nodes. One reactor had an additional lesion in the head only. Laboratory work completed to date reveals that on the basis of biochemical reactions the isolant from each of eight animals was characteristic of *M. avium* or Runyon Group II mycobacteria. Passage of the isolant from one of the eight through laboratory animals proved the organism to be pathogenic for chickens thus a classification of *M. avium*.

All prior herd tests were negative. The most recent was four years prior to finding this infection.

Most of the cattle in this dairy herd were reported as raised on the farm. All of those with macroscopic lesions were two years of age or less and were in the farm raised group.

Poultry and swine had been maintained on the premises but had been removed approximately six months prior to this test. Many rats as well as pigeons were reported on the premises. An acid-fast organism was isolated at the local laboratory from a sparrow.

This case is cited as unusual from the viewpoint of herds reported in which several animals were found with mesenteric lesions only. More extensive epidemiological investigations taking into consideration the laboratory findings of this and similar cases are bound to reveal additional information to aid in program planning. We are in need of more definitive
information relative to the significance and prevalence of atypical acid-fast organisms in domestic animals. This is particularly true of the Runyon Group III that are classed separate from *M. avium* by the fact that they do not produce macroscopic lesions in chickens.

Many of those here were privileged to hear the talk by Quinn and Towar at the American Veterinary Medical Association Convention in New York concerning tuberculosis in a herd of deer. For those who did not hear that talk, I'd like to summarize the case.

In the fall of 1962, a practitioner reported a condition resembling tuberculosis in a deer from a deer park in this area. This had been brought to his attention by a veterinary student from Purdue University, who had a summer job at the deer park. There were many types of deer present, the largest group of which were spotted fallow deer.

A total of 170 deer were tested. Other species tested included five llamas, four reindeer, four donkeys, one pony and seven spider monkeys. There were twenty deer that responded to the tuberculin test. None of the species other than deer responded.

On post-mortem examination of the twenty reactors, twelve disclosed extensive and eight no-gross-lesions. A white fluid to caseous type lesion was the typical finding. In only one doe were calcified lesions present. Lesion specimens were cultured at Michigan State University and isolates were identified as *M. bovis*.

The carcasses were disposed of by incineration in a large sand pit. Wood and old automobile tires formed a base on which the carcasses were piled and soaked with fuel oil.

We believe this case provides considerable interest from the standpoint of the potential interstate spread of bovine tuberculosis in species of animals other than cattle and swine. The potential public health hazard to the viewing public is also of interest.

In 1960 Karlson pointed out the serious hazard due to bovine tuberculosis in exotic animals in zoological gardens. His laboratory has isolated bovine tubercle bacilli from such animals as the giraffe, bison, elk, rhinoceros, eland, okapi, coatimundi, cheetah, sapajou, tapir, guanaco, peccary, orangutan, and monkeys of many kinds. This latter group is significant in that many people think of monkeys as being infected with *M. tuberculosis*. Obviously, from Karlson's report this is not always true and *M. bovis* must be considered when tuberculosis in monkeys is encountered.

Laboratory diagnostic services are invaluable to the program. While there are many questions still unanswered, the increased understanding gained is helping to guide our efforts into the most productive areas. The results of laboratory findings of specimens submitted for tuberculosis examination for fiscal year 1963 are summarized in Figure 2. Additional information relative to the laboratory work at NADL on tissues suspected of being tuberculous is covered in another paper at this meeting.

We have a recurring problem in our program that is frequently overlooked but is responsible for a delay in reaching our goal of eradication. This problem is best defined by a case history. The case was initiated by a report that an animal showed tuberculous lesions on ragular-kill. As a
result of traceback the originating herd was tested and a large number of reactors was disclosed. Meat inspection examination revealed that a high percentage of the reactors had lesions, including several condemned carcasses. In studying the history of this herd it was found that there had been a report several years earlier of reactors in another herd traced to the herd in question. However, no test was made at that time because it was assumed that the results of a recent area test gave assurance that the herd was free of infection. In some similar cases the routine test is not even recent. Even though there is little bovine tuberculosis left in the United States today we cannot allow ourselves to overlook any good leads as to where the infection is being nurtured. Any report of infection traced to a herd demands the retest of that herd immediately. The testing veterinarian must have the latest information available to form his professional judgment. A small response in one or two animals may be interpreted as non-specific by many veterinarians when no history is available. This is less likely to occur if the veterinarian is aware of associated tuberculous animals.

The report of the Status of State-Federal Bovine Tuberculosis Eradication last year referred to several recommendations of the Committee on Tuberculosis of this Association regarding the development of animal identification and carcass examination as an aid to eradication.
The identification and tracing of tuberculous animals has been recognized as a valuable program aid for more than half a century. We are making progress in adopting procedures recommended as far back as 1910 by the International Commission on the Control of Bovine Tuberculosis, from which I quote:

"This Commission recognizes that the discovery of tuberculosis in animals slaughtered for food purposes furnishes one of the best possible means of locating the disease on the farm, and therefore recommends the adoption of some system of marking, for purposes of identification, all cattle three years old and over, shipped to slaughter."

The importance of animal identification through slaughter is documented by the results of tracing reports for two states as illustrated in Figure 3.

<table>
<thead>
<tr>
<th>STATE</th>
<th>IDENTIFICATION</th>
<th>TEST RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO. CASES ADE 6-35</td>
<td>EAR TAGS</td>
</tr>
<tr>
<td>1.</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td>18</td>
<td>1</td>
</tr>
</tbody>
</table>

**Two State Contrast**

Figure 3

In the two states where the number of tuberculous lesion cases reported on regular-­kill by meat inspection were about equal, a wide contrast of results is evident. In the state where considerable identification was available to identify carcasses at time of slaughter 38 herds with reactors were found as compared to one herd in the second state. The one case in the second state that resulted in finding an infected herd was the single case where descriptive identification (an identification tag number) was reported. It is also significant to observe that approximately 70 percent of the reactors disclosed in each state as a result of these investigations
were found to have macroscopic lesions of tuberculosis. One has reason to speculate as to the number of additional tuberculous herds that might have been located if adequate identification had been available for all cases reported.

The effectiveness of the ear tag in traceback work is evident when reviewing a large number of these cases. To illustrate this point Figure 4 depicts a typical case where the ear tag helped to locate an infected herd in spite of the fact that the animal had lost its identity with the herd of origin as far as the records of sale were concerned. Individual permanent animal identification with central records to identify the herd where the tag was applied is a key to effective traceback.

**EARTAG EFFECT IN TRACEBACK**

ADE 6-35

The efficacy of selected testing is apparent when reviewing the traceback studies of 455 tuberculous animals reported by meat inspectors on regular-kill. These cases resulted in locating 773 reactors of which 339 or 51.6 percent had macroscopic lesions, as compared to the fact that only 15 percent of the reactors found as a result of all other tests applied during the year had macroscopic lesions.

Improved measures for identification of cattle from farm through slaughter may be divided into two phases: (1) Tags, brands, or other
identifying marks applied that identify the animal enroute to the plant and, (2) methods for maintaining identity until meat inspection examination is completed.

Progress has been made in utilizing the backtag information available through the Market Cattle Testing Program to identify cattle into the plants. Using this information the State of Washington was able to re-accredit the first counties under Paragraph 13, of the present Uniform Methods & Rules. Much more work remains in this area to make broader use of slaughter records in reaccrediting counties.

It is interesting to note that approximately 1/3 of the cattle slaughtered under Federal Meat Inspection are slaughtered in plants where the hide is separated from the carcass before inspection is completed. In these plants, the three-part tag or other similar device is needed to maintain identity of carcasses. The promotion of this aid was stimulated following the recommendation of the Committee on Tuberculosis that a goal of December 31, 1963, be established for having the three-part tag in operation in all slaughter plants where its use is needed. Use of the three-part tag has been inaugurated in 22 of 92 slaughter plants. This device is scheduled for use in 38 additional plants. Delayed bleeding of backtagged cattle has also helped maintain identity as the backtag is then kept with the carcass until inspection is completed. Individual meat packers and meat packers organizations should be commended for their interest and steps taken in carcass identification as an aid to animal disease eradication.

Progress made in eradicating tuberculosis in herds, some of which have been infected for several years, is exemplified by the fact that the number reported in the "Red Flag" category has been reduced from 239 in 1960 to 34 in 1963. There has been a decline in the number of "Red Flag" herds each year since the first report in June 1960, as shown in Figure 5. The average period of infection for herds presently classed as "Red Flag" is 4-3/4 years.

As a result of testing 8.3 million cattle in 339,640 herds in fiscal year 1963, 222 herds with lesions indicative of \textit{M. bovis} infection were studied. This does not include herds with mesenteric or body gland lesions only, when there was no laboratory proof that \textit{M. bovis} was involved, nor does it include herds from which other slight lesions were found when the laboratory findings were not indicative of tuberculosis on histopathologic examination.

As shown in Figure 6, 52 percent of the total tests were made as a part of area work to locate 49 percent of the herds with lesions indicative of bovine tuberculosis. Only one percent of the total tests were made as a result of traceback to locate 29 percent of the total lesion herds. Tests for all other reasons excepting tests of quarantine herds accounted for 43 percent of the total tests to detect 22 percent of the new lesion herds. If adequate identification were available so that tuberculous animals could be traced, the number of lesion herds undoubtedly would be considerably higher than the 29 percent found as a result of traceback, with very little additional expense. The key to effective epidemiology is good identification.
Tuberculosis Eradication

RED FLAG HERD PROGRESS

239 HERDS

AREA TESTING

JULY 1960

JULY 1961

JULY 1962

JULY 1963

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Figure 5

Study of 222 Lesion Herds Located in 1963

<table>
<thead>
<tr>
<th></th>
<th>Area Testing</th>
<th>Traceback Testing (6-35 &amp; 6-4 Reports)</th>
<th>Other Testing*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Tested</td>
<td>8,394,790</td>
<td>4,320,975</td>
<td>76,163</td>
</tr>
<tr>
<td>Percent of Total</td>
<td>52</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>No. Lesion Herds</td>
<td>109</td>
<td>65</td>
<td>48</td>
</tr>
<tr>
<td>Percent of Total</td>
<td>49</td>
<td>29</td>
<td>22</td>
</tr>
</tbody>
</table>

*Does not include testing in quarantined herds.

Figure 6

Benefits can now be derived from the expanded use of Animal Identification Programs as an aid in qualifying areas for reaccreditation. The new "Uniform Systems for Program Records" developed by Administrative personnel of ADE, available to all states, provide effective and practical
procedures for maintaining records for this phase of the program. This includes the development of statistical data and the maintenance of a record of cattle slaughtered at accredited establishments that are identified to the originating herds. A number of states are taking advantage of these procedures to reduce the number of herds required to be tested. As this phase of the program grows, costs can be reduced especially in those areas where no bovine tuberculosis has been reported for many years.

It is generally agreed that it is impractical to test all of the 115,000,000 cattle in this country every six years. Approximately 90 percent of the slaughter cattle are going to plants where effective meat inspection service is maintained. We need to take full advantage of this fact. We must promote systems whereby all cattle with tuberculous lesions are detected and traceable to their herds of origin.

It would be poor policy to curtail area testing in those areas with a relatively high incidence of bovine tuberculosis until it is shown that we can advance toward eradication using less costly techniques. It should again be noted, Figure 6, that approximately one-half of the total lesion herds are located as a result of area testing. Detecting early cases of tuberculosis promptly is preventing many herd owners from suffering the extensive losses encountered when a herd is riddled with tuberculosis.

We may not be able to get them all tested, but we have the capability of inspecting a high percentage of the animals at slaughter. Thus we stand at the "pearly gates" and check them off on their way through.

REFERENCES

REPORT OF THE COMMITTEE ON TUBERCULOSIS


Again this year the Tuberculosis Committee has held mid-year meetings. The entire Committee met in Chicago during March, and the Subcommittee on Paratuberculosis met in Ames during September. Public hearings in Albuquerque, however, were disappointing in attendance. Dr. M. Hynes of Dublin, Ireland outlined in detail the Irish tuberculosis program. This country has apparently solved many problems, including animal identification. They are to be congratulated.

The Meat Inspection Division of the ARS, USDA and the meat packing industry are to be congratulated on the many contributions they have made through the years to the success of the tuberculosis eradication program.

During the past year, twenty-two packing plants started using the three-part tag, a procedure whereby animals may be identified through slaughter. Thirty-eight additional plants have indicated that they will soon follow this procedure.

State and Federal officials are reminded of the December 31, 1963 goal. They should encourage the remaining plants to use this system by that date.

During the past year regional meetings were held with clerical personnel to increase their understanding of the tuberculosis program and to discuss better ways of processing program data, particularly trace-back information. This effort has stimulated their activity and thinking and will undoubtedly produce far-reaching benefits to the program.

In order to develop increasing stimuli in tuberculosis eradication, this Committee highly recommends the inclusion of a four-hour program, directed specifically at tuberculosis and all phases of the program, to be held during the annual Regional USLSA meetings. In addition, it is also suggested that groups of adjoining states combine and develop similar technical one to two day programs for their regulatory veterinarians and other interested parties. Several of these meetings were held at the field level this year and critiques indicated high praise for the excellent information received and constructive criticism that was presented. Two-way communication is continually needed to improve our present program by receiving comments and suggestions from personnel who are in the field carrying out the eradication activities.
It is essential that all tuberculin used for testing cattle be produced and evaluated in a uniform manner. It may be recalled that during the early years of the program tuberculin used in the State-Federal cooperative program was produced in the laboratories of the United States Department of Agriculture. In recent years, the Department has obtained tuberculin under contract from commercial sources.

Contracts were originally awarded annually to a single producer at a time. Due to production problems that have occurred during recent months, the supply of acceptable tuberculin has been limited. The Department has now found it necessary to obtain tuberculin from three biological concerns.

The production and testing standards are specifically outlined in the contract specifications.

To insure that all tuberculin used for testing cattle for interstate shipment or otherwise will be of a uniform high standard, it is recommended that all tuberculin licensed and approved by the Agricultural Research Service, United States Department of Agriculture be produced and tested in accordance with the same specifications and standards as for contract tuberculin.

We are pleased to note the progress that is being made in research studies being conducted at Michigan State University and the National Animal Disease Laboratory. Diagnostic tests are being developed that will aid in detecting tuberculosis spreaders in red flag herds. A serum repository of serums for tuberculin reactors is being established for use in serological studies.

We encourage the continued study by histopathologic and bacteriologic methods of tissues from tuberculin reactors and lesions from routinely slaughtered cattle suspected to be tuberculous, in order to provide more specific differential diagnoses of bovine tuberculosis from other granulomatous diseases.

We would also encourage regulatory officials to continue permitting research personnel to study special tests in problem herds under proper control.

Animal identification has always been an integral part of all animal disease eradication programs. In the eradication of tuberculosis it is now obvious from statistical studies of the Committee that the total sample of the cattle population must be increased, and yet continuous farm-to-farm testing of randomly selected negative herds is illogical. Examination of animal carcasses in packing plants with a traceback of lesion cases is a satisfactory solution to this problem, but its success is contingent upon satisfactory animal identification.

The future of all animal disease eradication programs is directly dependent on animal identification; the benefits are obvious, not only to farmers and ranchers, but also to all allied industries; and support will be forthcoming from the meat packing, the marketing and the transportation industries, as well as beef and dairy organizations, cooperatives, veterinary organizations, and similar organizations interested in the welfare of the animal industry. Western brand inspections agencies have already expressed a desire for national coordination. With the obvious need and
the variety of interested groups, the only factor lacking is that of a co-
ordinating leader. It is therefore resolved that the United States Livestock
Sanitary Association assume this obligation of leadership and bring all
groups together to work toward a satisfactory solution to the many details
necessary to bring about a complete, practical, and acceptable national
system of animal identification.

Part IV. Areas Accredited Free of Bovine Tuberculosis in the Domestic
Bovine

A state, a county or a block of several counties, which is part of a
progressive plan for complete state coverage may be declared Bovine Tu-
berculosis Free in the Domestic Bovine if the State complies with all the
provisions of this part:

13. (a) During the three year period from effective date of this part
an area may be declared Bovine Tuberculosis Free in the Do-
mestic Bovine if during two or more reaccreditation periods
that cover at least twelve successive years, no Bovine Tu-
berculosis has been found.

or

(b) The cooperating State and Federal officials have declared an
intent to qualify an area for free status and have records show-
ing that during each of at least three successive years not less
than five percent of the cattle over two years of age, or fifteen
percent over a three year period have been identified from
each individual herd directly into an accredited establishment.
All cattle twenty-four months of age or over in herds that do
not meet this requirement shall be free of reactors to a tuber-
culin test applied within the qualifying three year period.

14. Any pathologic (granulmatous) lesion in the domestic bovine sus-
pected of being tuberculosis will be considered bovine tuberculosis unless
a satisfactory examination at an approved laboratory justifies a diagnosis
other than bovine-type tuberculosis.

15. In the event that tuberculosis is disclosed or suspected, the pro-
visions of the individual accredited herd plan that relate to testing, quaran-
tine, removal of reactors, cleaning, disinfection, sanitation and epidemi-
ology shall apply.

16. All Cattle in herds from which infected animals originate and cat-
tle within the area that have associated with those found to be infected shall
be tested promptly. Tuberculosis when found will be treated as an exotic
disease with a complete epidemiological investigation and every effort
made to assure the immediate elimination of tuberculosis from all species
of domestic livestock and poultry on the premises.

17. State laws and/or regulations are in effect that provide for the
testing of any animal or herd when deemed necessary by the State-Federal
Cooperating Officials.
18. Bovine tuberculosis found by Meat Inspection examination or otherwise in any bovine traceable to an Accredited Free area will be considered to have originated from that area unless satisfactory records are available to definitely show that the subject animal had originated from a specific place outside the Accredited Free area.

19. Dealer control laws or regulations that require the identification of cattle and records of transactions for each animal purchased and sold are enforced.

20. Cattle moved in channels of trade within a state having a free area shall be identified and recorded as to origin and destination at the first concentration point (dealer, livestock auction, stockyard, etc.) as follows:

(a) Cattle that return to the farm, including feeding cattle, to be identified by permanent official ear tag or be branded cattle for intrastate movement only when accompanied by an official brand release.

(b) Cattle that are marketed for immediate slaughter shall be identified by permanent ear tag, sales tag, official back tag or a direct shipment of cattle accompanied by an official brand release.

21. Cattle moved for purposes other than for immediate slaughter into areas accredited free of bovine tuberculosis in the domestic bovine or areas in the process of attaining this status shall meet one of the following requirements.

(a) Cattle properly identified and moved directly into the Free area from another Accredited Free area.

(b) Cattle properly identified and moved directly into the Free area from a tuberculosis-accredited herd.

(c) Cattle from a modified accredited area that are properly identified and moved directly from a herd not under quarantine that have individually passed a negative test within thirty days of entering the premises.

(d) Feeder cattle from a modified accredited area properly identified and recorded as to herd of origin and maintained under quarantine until slaughtered at an accredited establishment.

22. No animal that has reacted or is suspicious to a tuberculin test or is otherwise suspected of being infected with bovine tuberculosis shall be moved into an area accredited Free of bovine tuberculosis for any purpose except direct to slaughter accompanied by an official permit.

23. All commercial slaughtering establishments in the area shall qualify as accredited establishments, except that those where not more than fifty thousand pounds of cattle (on a live-weight basis) are slaughtered in any calendar week may be exempt.

24. Disclosure of extensive or spreading bovine tuberculosis in a bovine tuberculosis free area and/or failure to take active steps to eliminate
any tuberculosis found shall constitute sufficient cause for revocation of
the tuberculosis free status.

25. Unless an area is disqualified, it may maintain its status of Bovine
Tuberculosis free in the Domestic Bovine for a period of six years pro-
vided that during this six year period five percent of the cattle over two
years of age or thirty percent over a six year period have been identified
from each individual herd direct to an accredited establishment. All cat-
tle twenty-four months of age or over in herds that do not meet this re-
quirement shall be subject to a tuberculin test applied within the requalify-
ing six year period.

The addition of Part IV, Areas Accredited Free of Tuberculosis, is
considered necessary by the Committee. This addition, however, must not
detract attention from the priority items as adopted by this Association in
1961 and approved by the USDA.

The following additions or corrections are recommended:

Part 1 - Definitions

(4) An "Accredited establishment" is one at which supervised meat
is maintained at all hours when slaughter is in progress and post-
mortem procedures meet recognized standards for the disclosure
of lesions of tuberculosis and the procedures are adequate for
maintaining the identity of individual cattle until inspection is com-
pleted.

Part 2

(1b) Herds in which reactors occur shall be quarantined and must pass
a negative tuberculin test after a period of at least sixty days to be
released from quarantine. If there are pathologic lesions considered
bovine tuberculosis in one or more reactors or in lesions of non-
reactors found at slaughter in animals traceable directly to the herd,
at least two negative tests at intervals of not less than sixty days will
be required for release of quarantine.

(1c) All herds in which bovine tuberculosis occurs shall be retested in
approximately twelve months but not more than fifteen months follow-
ing the first negative test after disclosure of reactors. At this time,
the herd may be accredited or reaccredited if it otherwise qualifies.
These tests are to be followed by at least two annual herd tests.

(6) Herd additions must originate directly from one of the following:
(a) Tuberculosis-accredited herd. (b) An area accredited free of
bovine tuberculosis in the domestic bovine. (c) A herd (not under
quarantine) in a modified accredited area tested and found negative
within twelve months. (d) Herds (not under quarantine) in modified
accredited areas if the individual animals pass a negative test within
thirty days of entering the premises.

Part 3

Delete Paragraph 12 and re-number Paragraph 13 as Paragraph 12.
REPORT OF THE SUBCOMMITTEE ON JOHNE'S DISEASE

Aubrey B. Larsen, Ames, Iowa, Chairman; Jack B. Flint, St. Paul, Minnesota; E. L. Brower, Trenton, New Jersey

The results of a national survey conducted by this subcommittee showed the following: (1) That Johne's disease is a problem in fourteen states. (2) That nineteen states reported the disease in cattle, sheep or goats in 1962. (3) There is a complete lack of uniformity among the states regarding the procedures used in infected herds.

It is recommended that vaccination of infected sheep on farms or ranches be conducted by the Agricultural Research Service in cooperation with state regulatory officials under controlled conditions on an experimental basis. For the present the vaccination of cattle against Johne's disease should be restricted to research institutions working with the disease.

It is recommended that more research be conducted on Johne's disease, particularly in the area of diagnosis, giving special consideration to the concentration and cultivation of fecal material, serological tests and the development of a uniform intradermal test.

Due to lack of uniformity of regulations between states it is recommended that in herds harboring infected animals if in the best judgment of the regulatory officials it is not practical to quarantine the entire herd, that all clinical cases, all animals showing typical bacilli on fecal smear and all animals showing reactions classed as positive and suspect to the intradermal test be quarantined.

It is recommended that tests used to diagnose Johne's disease, similar to those used in Canada, be required for cattle imported into the United States from all countries except Canada and Mexico.

It is recommended that close liaison be maintained between the Canadian Department of Agriculture, Health of Animals Branch and the U.S. Department of Agriculture, ARS in regard to research efforts and diagnostic procedures related to Johne's disease.

Since the control of Johne's disease presents a definite problem in the United States it is recommended that this subcommittee be continued.
DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS IN LYMPH NODES OF CATTLE THROUGHOUT COURSE OF INFECTION


SUMMARY

The foot-and-mouth disease virus (FMDV) content of lymph nodes was studied throughout the course of infection in cattle. Virus was detected in pooled samples of the large, main, lymph nodes, which usually are included with beef carcasses, from as early as 12 hours to as long as 15 days after inoculation. Thus, before clinical signs and obvious lesions appear and later when only scars of healed lesions are found, lymph nodes may contain virus. Virus was found in head lymph nodes as early as eight hours after inoculation and probably could have been detected much earlier. During the acute stage of infection, virus was found in nearly all lymph nodes examined. The average virus titer of prescapular lymph nodes from 32 cattle two to four days after inoculation was $10^{3.8}$ and the highest titer was $10^{5.7}$ mouse LD$_{50}$/gm. The titers were equal to or lower than the highest viremia titers, which may indicate that very little, if any, virus multiplication occurred in lymph nodes. However, virus persisted in lymph nodes longer than in blood. About four to five days after infection, the virus titers in both head and body lymph nodes began to decline coincident with the advent and rise in titer of neutralizing antibodies. At two days post challenge, virus was not found in lymph nodes of a recovered steer, reinoculated with FMDV of the homologous strain 43 days after initial infection.

INTRODUCTION

The lymph nodes of cattle are the main anatomical sites where foot-and-mouth disease virus (FMDV) may survive to present a hazard in boned meat offered for international trade. Thus, there is a practical need to determine the approximate times of appearance and disappearance and the titer of FMDV in lymph nodes.

Henderson and Brooksby\textsuperscript{1} reported on the survival of FMDV in lymph nodes of fresh and frozen beef carcasses and found titers as high as $10^{3.4}$ bovine ID$_{50}$/gm. Cottral \textit{et al.}\textsuperscript{2} demonstrated virus survival in lymph nodes of cured and uncured boned meat for as long as 50 days and in nodes

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**Laboratories AFTA, Moreno, Buenos Aires, Argentina.

The authors acknowledge the technical assistance of Messrs, J. Parker, D. Zaveski, E. Kramer, W. Parrish and A. Quintana.
of carcasses for as long as 60 days. Cox et al. found FMDV in a pre-
scapular lymph node surgically removed from a steer 20 hours post in-
oculation (HPI) before clinical signs and lesions were apparent. They also
found FMDV in lymph nodes for as long as nine days post inoculation
(DPI) in a convalescent steer.

Recently, Savi et al. reported on survival of FMDV in lymph nodes of
pigs and calves, and Uhlmann studied virus survival in lymph nodes of
adult cattle and swine. Virus titers in lymph nodes of cattle as high as
10^3.5 TC ID_{50} were reported by Wisniewski.6

Earlier studies related to the problem of FMDV in meat were men-
tioned in a previous review, but there were several reports that were un-
tentionally omitted.7,8 The importance of FMDV to the international
trade in meat was recently summarized by Moosbrugger and Brooksby.10
The political and economic aspects of the problem were reviewed by
Peffer.11

MATERIALS AND METHODS

Viruses

The seven known types of FMDV, A, O, C, SAT-1, SAT-2, SAT-3, and
Asia-1, were represented by strains designated by the British* as 119,
M 11, 149, RV-11, RHO-1, RV-7, and PAK-1, respectively. All viruses
had been maintained only by passage in cattle, from which infected tongue
epithelial tissue was harvested and stored at -50 C. For use, the tongue
tissue was ground with alundum and diluted by weight 1:10 with tryptose
phosphate broth of pH 7.4. After centrifugation at 880xg for 20 minutes,
the supernatant fluid was collected and penicillin G sodium, 10,000 units,
and dihydrostreptomycin, 10 mg., were added for each milliliter of the
virus suspension.

Virus titrations were made in Rockefeller H strain, Swiss suckling
mice, and virus dosages for cattle were calculated in mouse LD_{50} units by
methods previously described.12 The Henderson method was used for the
virus titrations in cattle.

Cattle

Grade Hereford steers were used. They averaged about 700 lb. and
were 18 to 22 months old. The method of management has been previously
reported.2 All donors of lymph nodes, except one as noted, were inoculated
in tongue epithelium, distributing the virus suspensions in about 20 sites.
All recipients of lymph node material, except those used for titrations,
were inoculated with two ml. of the material distributed in tongue epitheli-
um and five ml. via IM route. All cattle refractory to initial inoculation
were held 13 to 17 DPI. Then a blood sample was taken for serological
examination and intramuscular (IM) challenge inoculation of five ml. of
homologous virus was given. The challenge virus dosage was calculated
in each instance. Cattle resistant to challenge inoculation were observed

*Research Institute, Pirbright, Surrey, England.
for 10 days before slaughter. Post mortem examinations were made on all cattle.

*Lymph Node Samples*

The lymph nodes used were as follows: from the head - mandibular, suprapharyngeal, atlantal and paratid; from the body (associated with musculature and fat of carcass) - prescapular, prefemoral, internal iliac, ischiatic and popliteal. The lymph nodes were trimmed of fat, weighed and then minced with scissors. They were then ground in a mortar with a alundum and diluted by weight either 1:2 or 1:5 with tryptose phosphate broth of pH 7.4. The suspension of tissue was then either filtered through two layers of cheese cloth or was centrifuged at 880xg for 20 minutes to obtain the supernatant fluid. Penicillin G sodium, 10,000 units, and dihydrostreptomycin, 10 mg., were added for each ml. of fluid. Comparative tests resulted in similar titers with the two techniques, but centrifugation was preferred because alundum particles were eliminated. The cloth filtration technique was used for all samples referred to in Tables II and III.

For some of the tests, separate pools were made of the head and body lymph nodes. In others, the lymph nodes were tested individually. Titrations were made in suckling mice and in one experiment in cattle.

*Serological Tests*

The constant serum/variable virus dilutions technique was used for neutralization tests. All serums were heated at 56 C for 30 minutes and were diluted 1:10 with broth. Ten-fold virus dilutions were used and the serum and virus mixtures were incubated at 37 C for one hour. The tests were performed in suckling mice. Additional details and interpretation of tests were reported by Cottral et al.12

The technique of Siebold et al.14 was used for the fluorescent antibody examination of serums. In a previous study,12 it was found that this technique may be useful in detecting those animals which have had inapparent as well as clinically apparent lesions of FMD.

**RESULTS**

*Preclinical and early clinical stages of FMD.*—Six steers were inoculated in tongue epithelium, placing in each of 20 sites about 100 thousand mouse LD50 units of FMDV, A-119. Slaughter of the steers was arranged so that head and body lymph nodes could be harvested at eight, 10, 11, 12, 16 and 36 hours postinoculation (HPI). Samples of tongue epithelium from the sites of inoculation and heparinized blood samples also were taken.

Steers slaughtered at eight, 10, and 11 HPI did not have clinical signs or lesions of FMD. The steer slaughtered at 12 HPI had only three of 20 sites with minute, beginning tongue lesions and those at 16 and 36 HPI had both clinical signs and lesions. FMDV was found in head lymph nodes of all six steers and the titer ranged from 10^2.0 to 10^4.7 mouse LD50/gm. (Table I). Viremia was consistently found and virus was present in tongue epithelium of all six steers, even though lesions were not apparent in the first three slaughtered.
### Table I

Foot-and-Mouth Disease Virus (A-119) in Head and Body Lymph Nodes, Blood, and Tongue Epithelium of Cattle During Early Stages of Infection

<table>
<thead>
<tr>
<th>Hours Post Inoculation</th>
<th>Head Lymph Nodes</th>
<th>Body Lymph Nodes</th>
<th>Blood</th>
<th>Tongue Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detection</td>
<td>Titer</td>
<td>Detection</td>
<td>Titer</td>
</tr>
<tr>
<td>8</td>
<td>2/2**</td>
<td>3.0</td>
<td>0/2</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>2/2</td>
<td>3.4</td>
<td>0/2</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>2/2</td>
<td>3.4</td>
<td>0/2</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>2/2</td>
<td>4.7</td>
<td>2/2</td>
<td>1.5</td>
</tr>
<tr>
<td>16</td>
<td>2/2</td>
<td>2.0</td>
<td>2/2</td>
<td>1.8</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*First three donors did not have clinical signs or lesions of FMD, fourth donor (12 HPI) had only minute, beginning tongue lesions and last two donors had both signs and lesions of FMD. The tongue epithelium was site of inoculation.

**Number of steers infected/number of steers inoculated; N = virus not detected; - = no data.

Virus was not detected in the body lymph nodes of the three steers slaughtered at eight, 10, and 11 HPI, but was found at 12 HPI and thereafter. The virus titers found in the body lymph nodes at 12, 16, and 36 HPI were $10^{1.5}$, $10^{1.8}$ and $10^{3.6}$ mouse LD$_{50}$/gm., respectively.

**Clinical stage of DMV.**—During the acute clinical stage of FMD, virus was found in nearly all lymph nodes examined. Prescapular lymph nodes were taken from 34 steers slaughtered two to four days after inoculation with FMDV, A-119, or O-M11. Virus was found in 32 (94 percent) of the samples. The highest titer found was $10^{5.7}$ and the average titer was $10^{3.8}$ mouse LD$_{50}$/gm.

Some of the larger head and body lymph nodes from five other steers were examined from two to four days after inoculation with FMDV, A-119. The following titers in log mouse LD$_{50}$/gm. were obtained: mandibular, 3.2, 3.5, 4.3, 5.6; suprarahygeal, 4.1, 4.7; atlantal, 3.2, 3.8; parotid, 1.8, 3.8; prescapular, 2.8, 4.4, 5.5; prefemoral, 2.9, 4.0; internal iliac, 3.7; ischiatic, 4.1; popliteal, 3.9.

Comparative virus titrations were made in both steers and suckling mice of pooled samples of both the head and body lymph nodes. The samples were taken from 11 steers from one to nine days after inoculation with FMDV, SAT-1, O-M11, or A-119 (Table II). Virus was detected in all head lymph node samples, except one at eight days post inoculation (DPI), and in all body lymph node samples, except one at seven DPI. Two other body lymph node samples (at seven and eight DPI) contained insufficient virus to infect the recipient steers, but apparently contained sufficient active or inactivated virus to produce prechallenge serum neutralization indexes (at 17 and 13 DPI) of 5.6 and 3.1, respectively, and to render the steers resistant to IM challenge inoculation with 0.8 and 3.0 million mouse
TABLE II
Titers of Foot-and-Mouth Disease Virus in Head and Body Lymph Nodes of Cattle for Nine Days after Inoculation

<table>
<thead>
<tr>
<th>Days Post Inoculation</th>
<th>Virus</th>
<th>Head Lymph Nodes</th>
<th>Body Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ID50</td>
<td>LD50</td>
</tr>
<tr>
<td>1</td>
<td>SAT-1</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>O-M11</td>
<td>3.3</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>O-M11</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>SAT-1</td>
<td>3.6</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>O-M11</td>
<td>2.9</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>SAT-1</td>
<td>3.8</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>O-M11</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>A-119</td>
<td>3.0</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>SAT-1</td>
<td>1.3</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>A-119</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>O-M11</td>
<td>2.8</td>
<td>N</td>
</tr>
</tbody>
</table>

N = Virus not detected; P = virus detected, but titration not readable; - = no data; P* = immunized. The recipient steers were resistant to i.m. challenge with 0.8 and 3.0 million mouse LD50 units of FMDV and had prechallenge serum neutralization indexes of 5.6 and 3.1, respectively, for the 7 and 8 days post inoculation body lymph node samples.

LD50 units of FMDV, A-119. The fluorescent antibody reactions of these serums were negative.

The virus titers ranged from 10^{1.3} to 10^{3.8} bovine ID50/gm. and from 10^{1.3} to 10^{4.5} mouse LD50/gm. In two samples of both head and body lymph nodes, the mouse titers were higher than the cattle titers (O-M11 and SAT-1). However, virus was not detected by the use of mice after five DPI.

*Convalescent stage of FMD.*—Representative strains of all seven types of FMDV were used to infect cattle via tongue epithelium to determine the approximate time of disappearance of FMDV from the body lymph nodes. All of the cattle developed FMD within 24 hours after inoculation and had typical clinical signs and lesions. By the ninth day after inoculation, all were past the acute stage of infection. The cattle were slaughtered nine, 11, 13, 15 and 17 DPI. From each animal, pooled lymph node samples each were inoculated into two steers. The dose was two ml. in tongue epithelium and five ml. intramuscularly.

Virus was found in all five lymph node samples tested at nine DPI and in three of five samples tested at 11 DPI (Table III). Samples representing all seven types of FMDV were examined at 13 DPI and four were considered positive. At 15 DPI, only one of three samples was considered positive, and at 17 DPI, the three samples tested were negative. Four of the samples were listed as positive as a result of the serological and challenge response of the steers. One steer in each of the pairs that received the 13 and 15 DPI A-119 and the 11 DPI O-M11 lymph node samples resisted IM challenge and had a significant prechallenge serum neutralization index (i.e., 3.0 or more). Both steers that received the 13 DPI C-149
TABLE III
Foot-and-Mouth Disease Virus in Body Lymph Nodes of Cattle During Convalescent Stage of Infection

<table>
<thead>
<tr>
<th>Virus</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-119</td>
<td>2/2</td>
<td>1/2</td>
<td>0/2*</td>
<td>0/2*</td>
<td>0/2</td>
</tr>
<tr>
<td>O-M11</td>
<td>2/2</td>
<td>0/2*</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>C-149</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2*</td>
<td>0/2*</td>
<td>0/2</td>
</tr>
<tr>
<td>SAT-1</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>SAT-2</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>SAT-3</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Asia-1</td>
<td>1/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

A-119

*Indicates that one steer of pair was resistant to IM challenge and the corresponding significant neutralization index (NI) of the steer's serum. A NI of 3.0 or more is considered significant. Thus, these 3 steers apparently were immunized by the lymph node material.

**Indicates both steers were resistant to challenge and the corresponding NI of their serums. Positive fluorescent antibody reactions were obtained only with these two serums, probably indicating a latent infection of the two steers. All steers other than the 5 marked, as above, were susceptible to challenge and had negative serological findings.

Corresponding Neutralization Indexes of Prechallenge Serums from Steers Refractory to Initial Inoculation

<table>
<thead>
<tr>
<th>Virus</th>
<th>Days Post Inoculation</th>
<th>9/11</th>
<th>13</th>
<th>15/17</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-119</td>
<td>1.1; 3.7*</td>
<td>1.2; 3.1*</td>
<td>0.9; 1.4</td>
<td></td>
</tr>
<tr>
<td>O-M11</td>
<td>1.4; 3.1*</td>
<td>1.0; 1.3</td>
<td>1.0; 1.2</td>
<td></td>
</tr>
<tr>
<td>C-149</td>
<td>1.8; 1.5</td>
<td>5.5**; 5.5**</td>
<td>1.2; 1.3</td>
<td></td>
</tr>
<tr>
<td>SAT-1</td>
<td></td>
<td>1.2; 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT-2</td>
<td></td>
<td>0.8; 1.6</td>
<td>1.1; 1.3</td>
<td></td>
</tr>
<tr>
<td>SAT-3</td>
<td>1.0; 1.2</td>
<td>1.1; 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia-1</td>
<td>1.0; 1.2</td>
<td>1.1; 2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2/2, etc. = number of steers infected/number of steers inoculated.

Viremia and Serology. —During the first two DPI, viremia titers usually were higher than or equal to the virus titers of lymph nodes when concurrently made. After two DPI, viremia titers declined. A steer, inoculated with FMDV A-119, was used to determine the fall in viremia titer and the rise in neutralizing antibody level. At one, two, three and four DPI, the titer of virus in the blood was (in log mouse LD₅₀/ml.) 4.9, 4.1,
and negative, respectively. At five, seven, nine and 11 DPI, the neutralization indexes of the serums were 4.2, 4.7, 6.2 and 6.6, respectively. In similar tests with other cattle, viremia twice was detected as long as five DPI and the neutralization indexes sometimes had discrepancies.

**Recovered stage of FMD.**—A steer was inoculated with about six million mouse LD<sub>50</sub> units of FMDV, A-119 via the IM route. Two days later, the steer had signs and lesions of FMD. After 43 DPI, the steer had recovered so that only scars remained of the tongue and foot lesions. At this time, the steer's serum had a neutralization index of 5.9 and the steer was reinoculated intramuscularly with about 100 million mouse LD<sub>50</sub> units of FMDV, A-119. No clinical signs or lesions of FMD were found when the steer was slaughtered two days later. Individual pooled samples of the head and body lymph nodes and hemal nodes were inoculated into steers. Virus was not recovered from any of the samples.

**DISCUSSION**

When cattle are infected with FMDV by tongue inoculation, virus soon begins multiplication in the tongue epithelium. A short eclipse phase, during which very little or no virus could be detected, has been reported. Viremia may be detected as early as three to four HPI. The lymph nodes that receive lymph directly from the tongue (suprathyroidal, atlantal and mandibular) probably are the first to become infected. Henderson found that India ink appears in prescapular lymph node within one minute after intracutaneous injection of the neck in cattle. Thus, the injected virus could reach the regional lymph nodes from the tongue in a very short time.

By eight HPI, tremendous quantities of virus are being produced by the tongue epithelium, the head lymph nodes have accumulated a considerable quantity of virus and the blood is carrying a large virus population. Yet, virus was not detected in the large body lymph nodes until about 12 HPI. Thus, it appears that these nodes do not become infected until their afferent lymphatic vessels have accumulated and transported virus to the nodes. For some of the lymph nodes, infection may be dependent upon virus production in secondary sites. The epithelium of feet and sometimes the rumen undoubtedly produce virus long before lesions are observed, the same as in tongue tissue. Thus, most of the lymph nodes may contain virus before the advent of any clinical signs or obvious lesions. This stage of infection of cattle would not be detected by usual meat inspection techniques.

Within 24 to 36 HPI, the body lymph nodes reach the virus titer plateau that had been attained by the head nodes within eight to 12 HPI. The virus titer of most lymph nodes apparently remains at a plateau level for about four to five days, and very little, if any, virus multiplication occurs within the nodes. A specific cytopathic effect of FMDV on lymph node cells has not been described. However, the lymph nodes of infected cattle seem to be larger than those of normal cattle.

The decline in the virus population of lymph nodes begins and progresses coincident with the advent and titer increase of neutralizing antibodies. At this same time, virus production may be declining in the
epithelial lesions. In cattle that develop FMD within 24 HPI and follow usual course of recovery, some residual virus may be detected in body lymph nodes for as long as 13 to 15 DPI. The virus survival time in DPI probably would be correspondingly altered in cases where longer incubation periods were found. Animals slaughtered at 11 to 13 days after infection, in some cases, may be sufficiently recovered from FMD so that only small scars would be found on the tongue and feet. Nevertheless, they could have infectious virus in the lymph nodes.

Infectious FMDV may persist in lymph nodes for at least eight days during the time when antibodies, at a significant level, are continuously present in the circulation. Furthermore, the virus probably stimulates antibody production within the lymph nodes. Yet the virus is sufficiently protected within the lymph nodes to delay the time of total extinction of the population. An intracellular location may offer some protection for the virus, but the actual protective process may be more subtle and may be associated with antibody formation. Fishman et al.\textsuperscript{18} demonstrated that antibody formation takes place in vitro in cultures of lymph node cells, but only after the antigen (rabbit RNA) was transferred from macrophages to the lymphocytic cells. Perhaps, it is an advantage to the host to have residual FMDV present in lymph nodes to stimulate antibody formation long after the main epithelial lesions have ceased virus production. Other tissues where FMDV apparently may persist for much longer periods of time include the kidneys\textsuperscript{19} and salivary glands,\textsuperscript{20} but, in these tissues, the virus may have been located in cells beyond contact with antibodies, as was suggested in regard to kidney tissue by Hess et al.\textsuperscript{21} Not many other tissues have been tested for persistence of FMDV, however, preliminary studies with active bone marrow from ribs of infected cattle indicate that infective virus may be demonstrated for only as long as about five DPI.

Five of the steers used in this study (Tables II and III) apparently were immunized by virus material in the lymph node samples. This may have been due to a small amount of active virus or to inactivated or altered virus in the samples. The serological and challenge response of these steers was interpreted as evidence that FMDV in some form was present in the lymph node samples. Lymph node tissue contains a substance which has the consistency and fluid retaining properties of a gel. This gel-like substance may have served as an adjuvant in the apparent immunization of these five cattle. The technique used favored retension of this substance in the inoculum.

After recovery from infection with a specific type of FMDV, cattle are usually resistant to reinfection with virus of the same type for a variable period of time, but are susceptible to other types of FMDV.\textsuperscript{22,23} However, certain variant strains of FMDV may infect cattle that are immune to other strains of the same type.\textsuperscript{23,24} Thus, during the period of active immunity after cattle have recovered from infection with a specific type of FMDV, infectious virus probably could not be detected in lymph nodes even when large doses of homologous virus were used for challenge. But with heterologous strains and types of FMDV, demonstrable reinfection of lymph nodes may result.
REFERENCES


FIELD TRIAL OF LIVE VIRUS VACCINATION PROCEDURE FOR PREVENTION OF VESICULAR STOMATITIS IN DAIRY CATTLE
III. EVALUATION OF EMERGENCY VACCINATION IN GEORGIA

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Madison, Wisconsin

A live vesicular stomatitis (VS) vaccine prepared and safety tested at the University of Wisconsin is presently being field tested in Panamanian dairy cattle. Use of this vaccine to protect lactating dairy cattle during an active epizootic of vesicular stomatitis was feasible, but had not been field tested. The following report describes the use of the VS vaccine in lactating animals and presents field data collected from vaccinated and non-vaccinated herds during the recent VS epizootic in Georgia.

EXPERIMENTAL PROCEDURE AND RESULTS

Intramuscular inoculation of lactating dairy cattle with live vesicular stomatitis New Jersey virus vaccine. The purpose of this experiment was to determine the effect of the live VSV vaccine in lactating dairy cattle. The vaccine used in this experiment was the 1963 preparation described in the preceding paper.³ Four lactating cows were inoculated intramuscularly with one ml. of vaccine virus containing approximately 10,000 CELD₅₀ and four lactating control cows were inoculated with one ml. of a placebo vaccine. Animals receiving the live vaccine were stationed alternately in the milking line with animals receiving the placebo. A de Laval milking machine was used and the teat cups were not dipped in antiseptic between cows. Milk production records were kept from four days before vaccination to eight days following vaccination. Body temperatures were recorded twice daily for a period of a week following animal inoculation. Milk samples were collected from each quarter of each animal prior to and 24 and 48 hours after administration of the vaccines. The Hotis test, leukocyte count and quantitative bacteriological plating was performed on each quarter milk sample of each cow in the experiment. The composite quarter samples from each cow at 24 and 48 hours after inoculation were also tested for presence or absence of virus. Blood was collected for virus isolation attempts at 24 and 48 hours after inoculation and saliva was collected from each animal 48 hours after vaccination and tested for virus. Serum samples were collected from each animal before inoculation and at weekly intervals afterward. The animals were examined daily for vesicles during the period of study.

Neither the animals receiving the live VS New Jersey vaccine nor those inoculated with the placebo had a significant change in body temperature or in milk production, in the Hotis test reaction, leukocyte count, or

*Present address: Biological Specialties Corporation, Middleton, Wisconsin.
in type or quantity of bacteria in the milk. Virus was not isolated from the blood, milk or saliva and vesicles or anorexia were not observed in any of the animals.

The sera were evaluated in a tissue culture colorimetric neutralization test. The preinoculation serum sample of one animal had demonstrable VS New Jersey virus neutralizing (VN) antibody. The four animals inoculated with the live virus vaccine had a significant increase in VN antibodies to VS virus New Jersey serotype. Specific VS New Jersey VN antibody was demonstrable seven days after vaccination. The four control animals remained negative throughout the study.

The intramuscularly inoculated vaccine virus was not spread from animal to animal by the milking process or saliva. The animals inoculated with the live VS vaccine did not exhibit any adverse effects and had a prompt immune response to VS virus New Jersey serotype.

**Titration of vesicular stomatitis virus on the tongue of susceptible and immune cattle.** Two 14 month old heifers were inoculated intramuscularly with one ml. of the VS New Jersey virus vaccine. Serum samples were collected at the time of vaccination. Twenty-eight days after vaccination the two vaccinated animals and two susceptible heifers were inoculated intradermally on the tongue in two sites with ten fold dilutions of a Georgia isolate of VS New Jersey virus. Serum samples were collected from each animal at the time of lingual inoculation and 14 days later.

In previous experiments (Unpublished data) it had been determined that by the intralingual method of inoculation in cattle the VS virus would titer two and one-half logs less in cattle than when assayed by the intracerebral route of inoculation in weanling mice. This stock of VS virus titered $10^{10.5}$ per ml. intracerebrally in weanling mice and $10^8$ per ml. intralingually in 14 month old heifers. This same VS virus stock was used to challenge the non-vaccinated and vaccinated animals. The VS virus titered $10^{6*}$ and $10^8$ per ml. respectively by intralingual inoculation in the two non-vaccinated animals. The serum neutralizing indices of the control animals were negative at the time of intralingual inoculation. The vaccinated cattle were challenged at the same time as the control animals. The vaccinated animal which had a serum neutralizing index greater than $10^3$ at the time of challenge had an animal protection index of $10^4$. The second vaccinated animal had a serum neutralizing index of $10^{2.5}$ at the time of intralingual challenge and had an animal protection index of $10^2$ when a heterologous isolate of VS virus New Jersey serotype was used as challenge material.

**FIELD TRIAL PROCEDURE AND RESULTS**

*Herds vaccinated before epizootic.* Twelve dairy herds were selected for vaccination in three Georgia counties where VS had occurred the previous year (Table I). Five of the herds in the field trial had had experience with

*This control animal had been inoculated six months before with the Indian type vesicular stomatitis virus.*
the disease based on herd history. Two hundred and eighty-one animals were vaccinated and 275 non-vaccinated animals were retained as controls in the twelve herds. Eighty-six percent of the pre-vaccination serum samples from the animals on farms that had a history of VS in 1961 or 1962 were positive for VS New Jersey VN antibodies and 24 percent of the animals had VS antibodies on farms with no history of VS. The 1963 vaccine preparation described previously was inoculated intramuscularly in alternate animals and preinoculation blood samples were collected from each animal during June 7, 8 and 10, 1963. The cooperating dairymen were requested to notify their district United States Department of Agriculture, Animal Disease Eradication veterinarian if VS occurred in their herd.

The first part of July, 1963 the owner of herd E reported that one of his animals, which was pastured about 15 miles from the milking herd, had developed vesicular stomatitis. The disease did not appear in the lactating animals. The owner reported that there had been two VS cases in his lactating animals in 1962 and that one cow had died. A necropsy was not performed on the animal.

During the latter part of July the owner of herd F reported VS in a heifer pastured about one and one-half miles from the main farm and also two lactating animals at the main farm. The three affected animals had not been vaccinated with the VS vaccine. Vesicular stomatitis had been reported in this herd in 1961 and 1962. The herd bordering farm K had a few cases of VS during the middle of August, but no cases were observed on farm K. None of the other herds vaccinated in June reported VS during the summer. Post vaccination serum samples were collected from these twelve herds in August, 1963.
Herds vaccinated during epizootic of vesicular stomatitis. At the beginning of the vesicular stomatitis epizootic in Georgia this year all of the lactating animals in early affected dairy herds that requested protection were bled and vaccinated and also a percentage of lactating animals in non-affected dairy herds requesting protection that were in counties were active cases of VS were occurring were bled and vaccinated. Beef and non-vaccinated dairy herds that were affected with VS were studied for comparison with VS affected-vaccinated dairy herds.

Ninety-four dairy herds were vaccinated in 12 counties of Georgia (Table II, Map 1). Sixteen of these herds had active cases of VS at the time of vaccination and nine herds had VS in animals after vaccination. Three thousand eight hundred and sixty dairy cattle were vaccinated and 902 were held as non-vaccinated controls.

The course of the VS epizootic in five non-vaccinated herds of cattle was reviewed with herd managers. Farm ZA contained a herd of about
**TABLE II**

Georgia Dairy Cattle Vaccinated During Vesicular Stomatitis Epizootic

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<th>County</th>
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<th>Date Vacc.</th>
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<td>RB</td>
<td>Coweta</td>
<td>1963</td>
<td>89%</td>
<td>7-30</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>RC</td>
<td>Harris</td>
<td>None</td>
<td>5%</td>
<td>7-30</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>RD</td>
<td>Spalding</td>
<td>None</td>
<td>0%</td>
<td>8-1</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>RE</td>
<td>Spalding</td>
<td>None</td>
<td>9%</td>
<td>8-1</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>RF</td>
<td>Lamar</td>
<td>None</td>
<td>4%</td>
<td>8-2</td>
<td>91</td>
<td>16</td>
</tr>
<tr>
<td>RX</td>
<td>Spalding</td>
<td>None</td>
<td>NB</td>
<td>7-31</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>WA</td>
<td>Meriwether</td>
<td>1963</td>
<td>13%</td>
<td>7-29</td>
<td>60</td>
<td>21</td>
</tr>
<tr>
<td>WB</td>
<td>Troup</td>
<td>None</td>
<td>17%</td>
<td>7-30</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>WC</td>
<td>Spalding</td>
<td>None</td>
<td>15%</td>
<td>8-1</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>WX</td>
<td>Harris</td>
<td>None</td>
<td>NB</td>
<td>7-30</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>WY</td>
<td>Spalding</td>
<td>None</td>
<td>NB</td>
<td>7-31</td>
<td>32</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 3,860 902

NT = Not tested
NB = Not bled

850 Black Angus beef cattle. Active cases of VS were treated with antibiotics and topical medicine over a two week interval and 20 percent of the herd animals were affected. Five percent of the 550 beef cattle were affected with VS on farm ZB in the two weeks preceding this history and VS was still active in the herd. Approximately 90 percent of the 60 dairy cattle on farm ZC were affected with VS over a period of five weeks and a majority of the animals had teat lesions. One hundred percent of the 60 animals on ZD farm were affected with VS within a week and this herd happened to be one of the first herds affected in Meriwether County this year. There were approximately 300 beef cattle on farm ZE of which 50 percent contracted VS during a period of four weeks.

Seven herds, which had VS affected animals at the time of vaccination, did not have any more cases after vaccination. Vesicular stomatitis
occurred in non-vaccinated control animals on four farms up to a week after vaccination, on two farms up to two weeks and for two and one-half weeks on one farm. Clinical VS occurred in vaccinated animals on eleven farms during the first week following vaccination. The last case of VS occurred in vaccinated animals on four farms eight, nine, 10, and 20 days respectively after inoculation with the vaccine. Five days after vaccination the last of 12 inoculated animals developed VS on farm AK. Twenty-eight VS cases developed in animals inoculated with the vaccine on farm FA and these were all afflicted with teat lesions. The milking machine teat cups were not dipped in an antiseptic solution between cows on farm FA and whereas, dipping the teat cups between cows was a common practice on other dairy farms in this region.

Milk records from individual cows from affected herds were not available from this years VS epizootic. Herd bulk tank records were available for a few herds. Graph 1 is a comparison of bulk tank records from a VS affected-non-vaccinated herd (farm ZC) with the records from a VS affected-vaccinated herd (farm AK). All of the lactating animals on farm AK were vaccinated five days after the first case of VS occurred in the herd. The percentage of immune animals prior to the first case of VS is not known for either herd. If the assumption could be made that a majority of the animals on farm AK would have become affected with VS had the vaccine not been administered, then it could be concluded from the limited data in Graph 1 that the VS vaccine prevented a marked loss in milk production when administered to a herd of dairy animals in the early stages of a VS epizootic.

Graph 1. Average Milk Production Per Day Per Cow from Two Vesicular Stomatitis Affected Dairy Herds.
The economic impact of vesicular stomatitis on the livestock industry and the nation has not been adequately evaluated. Besides the loss in milk production and body weight, mastitis may develop due to secondary invaders and death may even occur in a small percentage of the affected animals.¹ Medical expenses, as well as loss of feed, time and space for finishing cattle for market add to the loss. The factor of human health must also be considered, because VS does cause disease in the personnel that handle affected animals. Following the 1961 and 1962 epizootics of VS in the Southeastern United States, a few reports on economic loss incurred by livestock owners were submitted. For example, an estimated loss of $15,000 was reported for three combined dairy herds of 687 total dairy animals in 1961. After the 1962 epizootic one Georgia dairyman reported an estimated loss of $20,000; seventy of his 169 lactating animals had been affected.¹

Following the 1961 and especially the 1962 epizootic of VS in the Southeastern United States dairymen urgently requested some means of protecting their cattle against VS. A live VS virus vaccine was being field tested in replacement dairy cattle in Panama. A field trial was proposed for the VS vaccine in lactating dairy cattle in the Southeastern United States since the program that was being used in Panama did not fulfill the requirements for use of the vaccine during an epizootic. It had been shown that live VS virus vaccine did not produce any adverse effects in four lactating cattle. Vesicular stomatitis virus was not isolated from any of the body fluids tested from these animals and the VS virus did not spread to four non-vaccinated control animals. The vaccinated animals promptly developed VN antibodies to VS virus New Jersey serotype. These animals were not challenged with a heterologous isolate of VS New Jersey virus.

Two immune and two non-immune animals were challenged on the tongue with ten fold dilutions of a heterologous isolate of VS New Jersey virus to obtain preliminary data on the relative amount of virus the immune animals were protected against. The vaccinated animals had a greater animal protection index than the non-vaccinated control animals. Although there was considerable difference in responsiveness of individual animals there seemed to be an association between the protection test index and serum neutralization index.

Prior to the VS epizootic season in Northwestern Georgia twelve dairy herds were selected for vaccination on the basis of their disease history. Approximately 60 percent of the 556 animals in these herds had VS New Jersey VN antibodies. Pre-vaccination serum samples from 86 percent of the animals on the five farms that had a history of VS in 1962 were positive for VS New Jersey VN antibodies. On the seven farms with no record of VS, 24 percent of the animals had VS antibodies at the June, 1963 bleeding.

A portion of 82 dairy herds was vaccinated during the 1963 epizootic of VS in Georgia. Twenty-three percent of this cattle population (3836 animals tested) had demonstrable VS New Jersey VN antibodies prior to
vaccination. Thirty-eight percent of the cattle (517/1352) in herds affected with VS in July and August, 1963 had demonstrable VS New Jersey VN antibodies in July, 1963 and 15 percent of the cattle (375/2486) in non-VS-affected herds had demonstrable antibodies in July, 1963 prior to vaccination. The difference between the number of reactors in the composite samples represented by 38 percent and 15 percent is significant. However, there was considerable variation in the percentage of reactors in the individual herds. The greater number of reactors in herds that became affected in 1963 may reflect in part true antibody that developed in 1963, since some of the animals had become affected as long as a week to ten days before bleeding and vaccination. The greater number of reactors in the herds affected in 1963 as compared to the smaller number of non-affected herds may be a reflection of the difference in risk of these herds to infection with VS. The presence of animals with antibody in herds without a history of disease may be explained by occurrence of subclinical cases during the previous epizootic years or of a failure to recognize the disease in these animals.

In those instances in which individual animal records were available, the individuals that developed disease were serologically negative at the beginning of the epizootic. Thus the assumption is made that the animals that developed clinical VS were serologically negative prior to the disease. The following percentages were based on the animals that did not have VS antibodies prior to the use of the vaccine in the nine herds that developed VS after vaccination. Among the non-vaccinated control animals ten percent developed clinical VS and among the vaccinated animals in the same herds only two percent developed clinical disease. Vesicular stomatitis cases occurred in the non-vaccinated animals up to 18 days and in the vaccinated animals up to eight days after vaccination.

Twenty-six percent of the cattle in non-vaccinated herds developed clinical VS as compared to only five percent of all of the cattle vaccinated during the epizootic. Forty percent of the clinical cases occurred in the non-vaccinated herds during the first week and an additional 40 percent of the cases during the second week. In vaccinated cattle, 80 percent of the clinical VS cases occurred during the first seven days and only an additional 19 percent of the remaining cases had occurred by the end of the second week after vaccination. The final cases occurred in the non-vaccinated herds up to five weeks after the initial onset of the epizootic in the herd. Only one vaccinated animal (farm FA) developed clinical VS during the third week after it had been vaccinated.

From these data it can be concluded that the live VS New Jersey virus vaccine, administered intramuscularly during an epizootic, markedly reduced the number of clinical cases of VS in lactating dairy cattle. The period during which VS was present in the herd was markedly shortened following vaccination.
ACKNOWLEDGMENTS

The authors are indebted to Dr. C. J. Mike1 of the United States Department of Agriculture, Agriculture Research Service, Animal Disease Eradication Division, Atlanta, Georgia for organizing and directing the field trial in Georgia. Acknowledgment is also made to the State and Federal veterinarians and cooperating personnel for collecting blood, vaccinating and keeping herd records; to Mr. David Nassif for screening the thousands of sera in the colorimetric neutralization test; and to Biological Specialties Corporation for use of equipment and technical aid in preparation of the VS vaccine used in Panama and Georgia.

Financial support was supplied in part by the National Institutes of Health, United States Department of Agriculture and the Georgia State Department of Agriculture.

REFERENCES

FIELD TRIAL OF LIVE VIRUS VACCINATION PROCEDURE FOR PREVENTION OF VESICULAR STOMATITIS IN DAIRY CATTLE
II. SECOND YEAR EVALUATION IN PANAMA

Lloyd H. Lauerman, Jr., D.V.M., M.S. and Robert P. Hanson, Ph.D.

Madison, Wisconsin

A field trial of three years duration to evaluate the efficacy of a live vesicular stomatitis (VS) New Jersey virus vaccine administered intramuscularly to dairy cattle was begun in July, 1962 in Panama. In the following paragraphs information is presented on virus stability and stabilizers, preparation and application of the VS vaccine used in the 1963 field trial and evaluation of the data obtained from the pre and post vaccination serum samples.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of light, temperature and protective agents on vesicular stomatitis virus. To evaluate six organic compounds for their ability to protect virus in the fluid state the following experiment was designed. The compounds and their final percentage in solution are listed in Table I. Representative samples of each virus-compound were exposed to eight foot-candles of light from a common frosted light bulb and the other representative samples were held in total darkness. Half of the lighted and darkened samples were exposed to a temperature of 37 C and the other half of the samples were exposed at 23 C. The samples under each of the conditions were titrated for infective virus at zero, 48 and 96 hours. Casein hydrolysate gave the virus the best protection under the conditions of the experiment.

TABLE I

Effect of Physical Agents on Survival of Vesicular Stomatitis Virus

<table>
<thead>
<tr>
<th>Virus Protective Agents</th>
<th>Virus Detrimental Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light Temperature 37</td>
</tr>
<tr>
<td></td>
<td>48 96 48 96</td>
</tr>
<tr>
<td>0.75% Bovine Serum Alb.</td>
<td>5.5**</td>
</tr>
<tr>
<td>5% L-Cysteine</td>
<td>5.5</td>
</tr>
<tr>
<td>5% Caseine Hydrolysate</td>
<td>5.5</td>
</tr>
<tr>
<td>5% Bovine Gamma Globulin</td>
<td>5.5</td>
</tr>
<tr>
<td>2% Bovine Red Blood Cell</td>
<td>5.5</td>
</tr>
<tr>
<td>5% Hemoglobin</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Hours of Incubation
**Titer of virus expressed in log 10.

483
Vaccine preparation and application. The vaccine preparation consisted of one part of allantoic fluids from eleven day-old chicken embryos containing vesicular stomatitis virus, New Jersey serotype and three parts of a casein hydrolysate-sucrose solution. Two milliliters (ml.) of the virus-stabilizer mixture were dispensed into 10 ml. vaccine vials and rubber stoppers placed aseptically in the mouth of the vials. The vaccine was freeze-dried under vacuum in a Repp Sublimator model number 41 over a 24 hour period. The vials were sealed under vacuum and aluminum caps clamped on over the rubber stoppers. The vials were tested for vacuum by the use of a high frequency coil, which will detect a vacuum of 500 to 1000 microns of mercury. The lyophilized vaccine vials were stored at 4°C.

The Wisconsin Alumni Research Foundation analysed the lyophilized product for total moisture by the Karl-Fisher method and reported 4.19 and 4.14 percent total moisture of two representative samples. Graph 1 depicts the loss of virus in the lyophilized vaccine when subjected to constant temperatures. During exposure to constant 37°C temperature the lyophilized live virus preparation lost one-half log of infective virus the first week and did not loose in titer the remaining four weeks. One log of VS virus remained after ten minutes exposure to 100°C temperature. The identity, potency and safety tests were performed essentially as reported last year. The animal sera were screened for virus neutralizing (VN) antibodies in a colorimetric neutralization test described by Kuns.

Three hundred and twenty dry pregnant cows and 200 heifers, eight to 18 months of age, were vaccinated this year. Sixty-three heifers were retained as nonvaccinated controls. The dry cows ranged in age from three to 13 years of age and were eight to nine months pregnant. Each vaccinated animal received one ml. of reconstituted vaccine intramuscularly, containing approximately 10,000 CELD₅₀ of VS New Jersey virus. The vaccination took place in May during the inter-epizootic period.

Serological data. Data from 424 paired sera from animals on the field trial in 1962 paired sera from animals on the 1963 portion of the field trial are summarized in Table II. Fifty percent of the susceptible vaccinated animals developed VN antibody to VS New Jersey virus within the two weeks between vaccination and bleeding in 1962. Seventy-seven percent of the susceptible vaccinated animals developed VN antibody during the three week period following vaccination in 1963.

<table>
<thead>
<tr>
<th>Year</th>
<th>Before Vaccination</th>
<th>Post Vaccination</th>
<th>Vaccinated Group</th>
<th>Nonvaccinated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>-</td>
<td>-</td>
<td>145</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>146</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Total Paired Sera</td>
<td></td>
<td></td>
<td>312</td>
<td>112</td>
</tr>
<tr>
<td>1963</td>
<td>-</td>
<td>-</td>
<td>66</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>227</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>192</td>
<td>18</td>
</tr>
<tr>
<td>Total Paired Sera</td>
<td></td>
<td></td>
<td>485</td>
<td>165</td>
</tr>
</tbody>
</table>

Blood was collected in November, 1962 from a sample of the cattle on the vaccination trial. Table III summarizes the serological results from serum samples collected from the same animals on July 23 and 24, 1962 post vaccination with samples collected November 14, 1962. Vesicular stomatitis New Jersey VN antibodies could not be detected in the November serum samples of approximately 40 percent of the vaccinated animals that had developed VN antibodies in July, 1962. Forty percent of the vaccinated animals that had not developed antibody at the time of the two week

<table>
<thead>
<tr>
<th>Serological Reaction</th>
<th>July</th>
<th>November</th>
<th>Vaccinated Group</th>
<th>Nonvaccinated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td></td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>
post vaccination blood sampling in July had demonstrable VS VN antibodies at the November bleeding. Two of the 23 non-vaccinated control animals had converted to positive on the November sampling indicating that VS virus had been active in the area. Vesicular stomatitis had occurred in eight unvaccinated and one vaccinated lactating dairy cattle on the main farm by the middle of November, 1962.

**TABLE IV**

<table>
<thead>
<tr>
<th>Serological Reaction</th>
<th>Vaccinated Group</th>
<th>Nonvaccinated Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>1963</td>
<td>58</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>85</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>112</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Total 278</td>
</tr>
</tbody>
</table>

Table IV summarizes the serological data compiled from serum samples collected from animals on July 23 and 24, 1962 with samples collected in May 5 through 11, 1963, from the same animals. About 60 percent of the vaccinated animals that gave a positive serological reaction in 1962 did not have detectable antibody by the 1963 bleeding. Twelve of 88 non-vaccinated control animals converted to positive between the 1962 and 1963 bleeding.

A small percentage of the animals vaccinated in 1962 were revaccinated in 1963 and Table V summarizes the serological data from these animals. When these animals were vaccinated in 1962, twelve of 24 (50 percent) had a positive serological conversion by two weeks post vaccination. Only two of these positive animals had demonstrable VN antibodies before vaccination in 1963. Twenty of the 23 animals that gave a negative serological reaction before vaccination in 1963 converted to positive by three weeks after the second vaccination with the live New Jersey VS virus vaccine.

The serological data were separated as to age groups and compared with active VS cases reported each year as an index of virus activity (Table VI). The VS cases were only reported from the milking dairy cows.

**DISCUSSION**

The vaccine prepared in 1962 consisted of equal parts of allantoic fluids and 0.2 percent bovine serum albumin in buffered water. It was lyophilized in a Centrifugal Freeze Dryer model number 3 P.S./A. and sealed under nitrogen. Evaluation of the experimental data and field serological data indicated that the vaccine preparation used in 1962 was
TABLE V

Serological Data from Individual Animals Vaccinated Both Years

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5343</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5403</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>+</td>
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<tr>
<td>5456</td>
<td>-</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>5457</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5472</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5492</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>5529</td>
<td>-</td>
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<td>+</td>
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<td>5550</td>
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<td>+</td>
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<td>5577</td>
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<td>+</td>
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<tr>
<td>5610</td>
<td>-</td>
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<td>-</td>
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</tr>
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<td>5641</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>5670</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>5704</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5766</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5779</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5787</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5822</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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<td>5824</td>
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<td>+</td>
</tr>
<tr>
<td>5834</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5855</td>
<td>NT</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5868</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5893</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6019</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6337</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6563</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table Summary:

<table>
<thead>
<tr>
<th>12 negative - negative 1962</th>
<th>12 negative - positive 1962</th>
<th>26 negative 1963 before vaccination</th>
<th>2 positive 1963 before vaccination</th>
<th>3 negative 1963 post vaccination</th>
<th>22 positive 1963 post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Positive</td>
<td>- Negative</td>
<td>NT Not Tested</td>
<td>30 percent of the serum samples</td>
<td>30 percent of the serum samples</td>
<td>contained VS VN antibodies.</td>
</tr>
<tr>
<td>relatively instable and the virus dose was insufficient to produce immunity in greater than 50 percent of vaccinated animals within the two week post vaccination period. Four months after vaccination, serum samples collected during November, 1962, approximately 40 percent of the vaccinated animals that had developed VN antibodies in July had converted from positive to negative and approximately 40 percent of the vaccinated animals that had not developed VN antibodies in July had converted from negative to positive. The implication of these data are that ultimately perhaps up to 70 percent of the vaccinated animals developed antibodies to the VS vaccine virus. Ten months after the 1962 vaccination, 30 percent of the serum samples from inoculated animals contained VS VN antibodies.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE VI
Comparison of Serological Data in 1963 with Reported Vesicular Stomatitis Cases on Yearly Basis

<table>
<thead>
<tr>
<th>Year</th>
<th>VS Cases Reported</th>
<th>Age Group (Year)</th>
<th>No. Animals in Age Group</th>
<th>Serological Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Positive</td>
</tr>
<tr>
<td>1962</td>
<td>10</td>
<td>1</td>
<td>88</td>
<td>7</td>
</tr>
<tr>
<td>1961</td>
<td>40</td>
<td>2</td>
<td>214</td>
<td>34</td>
</tr>
<tr>
<td>1960</td>
<td>21</td>
<td>3</td>
<td>71</td>
<td>14</td>
</tr>
<tr>
<td>1959</td>
<td>53</td>
<td>4</td>
<td>71</td>
<td>7</td>
</tr>
<tr>
<td>1958</td>
<td>0</td>
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<td>1952</td>
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<td>7</td>
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<tr>
<td>1951</td>
<td>NR</td>
<td>12</td>
<td>38</td>
<td>21</td>
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</table>

NR = No Record

Vesicular stomatitis virus was active in the cattle population during the winter period as evidenced by the 13 percent conversion rate of the control animals (Table IV) and active cases of VS in the milking herd. The combination of a small percent of bovine serum albumin as a virus protectant and the introduction of nitrogen gas, which most likely also added moisture, were considered factors responsible for the vaccine virus instability.

During the months following the 1962 field trial, studies were conducted on means of improving stability in the fluid, as well as, in the lyophilized state. The final vaccine preparation used in the 1963 field trial consisted of one part allantoic fluids and three parts of a casein hydrolysate-sucrose stabilizer. The live virus vaccine was lyophilized in a Repp Sublimator and sealed under vacuum. Under much more rigorous test conditions it was demonstrated that the 1963 vaccine was more stable and a better immunizing preparation than the 1962 vaccine product. Seventy-seven percent of the susceptible animals vaccinated in 1963 had demonstrable VS New Jersey VN antibodies three weeks post vaccination. Ninety percent of the animals that were vaccinated in 1962 and revaccinated in 1963 had demonstrable antibody three weeks following the second injection.

In 1962 the delayed immune response to vaccination can be explained in part by the low number of infectious virus units used in the vaccine. The animals that were positive at two weeks and negative at four months may be indicators of a poor immunizing stimulus or may be examples of fluctuating titers. Sorensen, et al. reported marked fluctuations in VN antibody titers for VS of individual cows. Experimental inoculated cattle held in isolation yielded titers varying from $10^3 LD_{50}$ virus neutralized to less than $10^1 LD_{50}$ virus neutralized to $10^3$ again when successive samplings were made at intervals of two to four months. This phenomenon of an
inconsistent neutralizing index cannot be ignored in evaluating the duration of immunity in vaccinated animals.

Viral interference could be a problem in a VS vaccination program of cattle and could possibly explain the lack of responsiveness of a portion of the animals. Deinhardt and Henle\(^2\) have demonstrated that two days following infection with polyoma virus cultures of mouse embryo cells were resistant to VS virus. Dutcher\(^4\) reported that a number of tissue cultures derived from cattle with lymphosarcoma were found to be resistant to VS virus and lymph node tissue culture from a normal cow was susceptible to VS virus. Wagner and Snyder\(^8\) reported that L cells infected with lymphocytic choriomeningitis virus interfered with the cytopathic effect of VS virus. These are only a few examples where unrelated viruses have interfered with the growth of VS virus in tissue culture. Whether concomitant viruses impair the ability of the cattle to develop antibodies to the VS vaccine virus is unknown.

Sixty percent of the Panamanian cattle between one and two years of age had a demonstrable immune response after vaccination, whereas, eighty percent of the cattle over two years of age responded to vaccination. New born calves obtain maternal antibodies through the colostrum. Detectable maternal antibodies against VS persist in the calf four to six months. Cattle less than one year of age have rarely been reported with clinical signs of VS during epizootics.\(^1\) Six month old calves have a biphasic temperature rise after lingual exposure to VS, whereas, older animals have a monophasic temperature rise. Young cattle are apparently less susceptible or less responsive to VS than adult cattle as evidenced by absence of reported clinical disease and by significantly lower percentage of young animals that respond to vaccination.

In a personal communication Dr. Dreesen\(^3\) reported an abortion in a Georgia dairy herd that had active cases of VS during the 1961 epizootic and also similar circumstances in another dairy herd during the 1962 VS epizootic in Georgia. This information had been related to him by the farmers and the aborted feti were not available for study to determine the specific etiological agent. Safety test cattle in Wisconsin and Panamanian cattle in all stages of pregnancy have been inoculated intramuscularly with the VS vaccine without producing abortion. Of 144 recorded abortions occurring over a six year period in the Panamanian herd of approximately 2,000 animals only one animal had history of being infected with VS and that infection occurred one month after it had aborted. Vesicular stomatitis is seasonal in Panama, occurring during November, December and January, whereas, abortions are reported every month of the year in all stages of pregnancy. To the best of our knowledge abortions have not been reported in the literature as a sequelae of an epizootic of VS.

Vaccination of heifers between the age of eight to 18 months and again a few months before first calving appear to be an essential part of a program to protect lactating dairy cattle from the effects of VS. Judging from the available data, yearly re-vaccination of dairy cattle may be necessary to prevent loss produced by vesicular stomatitis virus.
REFERENCES


REPORT OF THE COMMITTEE ON VESICULAR DISEASES


CURRENT THREAT-FMD

The Committee on Vesicular Diseases is pleased to report that there has been an apparent improvement in the world-wide foot-and-mouth disease (FMD) situation since our October 1962 report to this organization. The contributions to this improved situation are many; and although efforts by certain countries have received much more attention than others, it must be said that combined effects of the less publicized efforts have contributed to this brightened picture.

Most notable, perhaps, is the result of the international cooperation which has apparently brought the spread of South African Territory Type I FMD (SAT I) under control in the Thrace (European) area of Turkey. You may recall the precarious situation facing the European countries at this time last year. The world-wide supply of type SAT I vaccine was very limited. Early contributions of funds from Ireland and Switzerland and donation of vaccine from Great Britain and Israel permitted early action to block further spread of SAT I FMD into Greece. These initial contributions were followed by contributions from Yugoslavia, Austria, and the Food and Agriculture Organization of the United Nations. The new diagnostic and vaccine production facilities established in the fall of 1962, through international cooperation must also be credited with a substantial contribution to the improved situation in Turkey.

By January of this year, sufficient vaccine to immunize 800,000 animals in Greece, 250,000 animals in Bulgaria, and 230,000 in Turkey had been made available to these three countries. This supply permitted the vaccination of animals along the Turkish border with Greece and ring vaccination of established foci of infection, in Turkey. The entire Greek province of Evros, bordering on Turkey, was also included in the buffer zone. A buffer zone was also established along the Bulgarian border with Turkey. This early action limited the extension of two outbreaks in Greece which were immediately brought under control by the Greek officials.

In Turkey, improved control procedures and a continuing vaccination program has limited the number of cases in the first six months of 1963 to six (6) in the Province of Kirklareli on the Bulgarian border and to one (1) case in the Province of Edirne on the Greek border. As for the other two provinces in European Turkey, Tekirdag has had eight cases since January 1, and Istanbul has had thirteen cases. The additional vaccine anticipated from the Etlik Institute should contribute significantly to the goal of the livestock officials to vaccinate 100 percent of the livestock in European
Turkey and extend ring vaccination to the ten (10) most western provinces of Asiatic Turkey.

In August 1961, France initiated a program of vaccination and systematic slaughter of infected animals. Since that time there has been marked decrease in FMD. There were 198 outbreaks in 1962 compared to 2,626 outbreaks in 1961 and 7,381 in 1960.\textsuperscript{13}

The Netherlands, Belgium and Denmark experienced several outbreaks of FMD in swine in late 1962 and early 1963. In spite of control measures, the disease continues to be a problem in the Netherlands. In Denmark, control measures have apparently been effective.

The last outbreak of FMD in Great Britain occurred in June 1962. This is the first time since 1918 that Great Britain has been free from the disease for more than 12 months. This may be a reflection of more effective control methods on the continent.

International efforts are being directed toward coordination of control and eradication programs in South America. Particularly severe outbreaks have occurred in Venezuela, Chile and Peru.

In Venezuela the important livestock producing States of Zulia and Apure have experienced severe outbreaks of FMD during the last 12 months. Livestock movements are restricted. Shipments to slaughter are the only movements permitted. Vaccination including the use of live virus vaccine has continued on a limited scale.

In Chile, the disease has been very serious. There was a shortage of available vaccine when the outbreak occurred. This experience re-emphasizes the importance of advance preparations and constant readiness. The outbreak in Central Peru is reported to be under control. Local vaccine supplies were exhausted requiring the procurement of vaccine from other countries. It will be some time before the total effect of control measures being applied in South America can be evaluated.

Recent, though unconfirmed, evidence has been considered sufficient for United States Department of Agriculture to add Cuba to the list of FMD infected countries. This development re-emphasizes the need for all individuals associated with the livestock industry to be ever vigilant for possible vesicular disease conditions and encourage the immediate reporting of such cases to appropriate officials.

VESICULAR STOMATITIS

There was an unusually severe outbreak of vesicular stomatitis (VS) in the United States during 1963. The number of confirmed cases was more than double the number reported in 1962, probably a result of the increased investigative staff assigned to the outbreak. The 1963 figures are believed more accurate than those reported for 1961 and 1962.

The apparent increase in the incidence of VS during the past three years, as reflected in the number of confirmed cases, is however, considered a valid increase in the incidence of the disease over previous years.
The first 1963 case occurred in a horse during the month of February in Coosa County, Alabama. Cases were confirmed in Berkeley and Marion counties, South Carolina in June. From July 1 through October 1, 415 vesicular disease investigations were made with 279 confirmed as New Jersey vesicular stomatitis (NJVS). Three hundred sixty-six (366) of these investigations were made in Georgia with 259 confirmed cases of NJVS occurring in sixteen counties. Fifteen of these counties are located in a group on the west central State line south and west of Atlanta. Lowndes County, on the southern border adjacent to Florida had one confirmed case.

The disease in Alabama has been more sporadic with 24 suspected cases investigated, revealing positive NJVS in sixteen cases in six counties. Three additional cases were confirmed in South Carolina and one positive case was diagnosed by serum neutralization test on serum submitted from Clark County, Arkansas.

On occasion, VS has spread to other parts of the United States; however, the 1963 outbreak was confined primarily to the southeastern States. During each of the last three years, VS has occurred on the northern fringe of the enzootic area. If VS is now established in that area, redefinition of the northern boundary of the enzootic area is indicated.

During the late spring and summer of 1963, an entomologist and an epidemiologist gathered additional information in the enzootic area in an attempt to determine the method of transmission of the disease. A report of their observations and conclusions will be reported at a later date.

Contrary to the usual picture of VS, many of the cases found during 1963 were sufficiently severe to cause heavy losses. A preliminary report carries an estimate of nearly 1/4 million dollars loss sustained by the livestock industry in Georgia. In addition to this loss, expenditures by the regulatory officials in Georgia have been estimated at more than $25,000. A detailed report of the economic aspects of the Georgia outbreak is being prepared and will be submitted to one of the veterinary journals for publication.

In early June, a field trial with an attenuated live virus vaccine was initiated in Georgia. The preliminary evaluation of this trial has been reported at this meeting by Dr. Lloyd Lauerman and Dr. R. P. Hanson.

The threat of a widespread outbreak of FMD in the United States is magnified by the regular occurrence of VS in this country. Complacency in the handling of a suspected vesicular disease condition, by assuming that the condition is VS, could be disastrous.

It is gratifying to report that, during the past season, livestock regulatory officials in the Southeast have been exceptionally alert, and have been diligent in initiating prompt investigations of suspected foreign animal diseases. However, one case of suspected vesicular disease, which occurred outside the enzootic area, was not reported to regulatory officials until seven days had passed following the appearance of the initial case. During that week, the infection spread in the herd until the morbidity exceeded 50 percent. This case was later confirmed as NJVS, which as you know is indistinguishable from FMD. Our efforts must be
intensified to secure immediate reporting of all cases of vesicular disease.

To meet our responsibilities in the detection, control, and eradication of an outbreak of FMD, a threat which will exist as long as the disease exists anywhere, the Committee recommends that this Association urge all segments of the industry to lend their support to the following areas of endeavor:

1. To obtain prompt reports of all suspect vesicular disease conditions. The immediate reporting of the case to appropriate livestock disease control officials could mean the difference between a prompt eradication or a long and costly control program.

2. To obtain full compliance with state laws and regulations controlling the feeding of garbage to swine. We are all cognizant of their value demonstrated in controlling the spread of vesicular exanthema.

3. To establish and finance a laboratory facility and epizootiological study in the enzootic area of southeastern United States to study vesicular stomatitis with particular emphasis on transmission and reservoir host. The establishment of such facilities is imperative to provide the knowledge necessary to obtain control of this economically important disease.

VESICULAR DISEASES - RESEARCH

**Vesicular Stomatitis**

In September 1961, a virus (Tr 40233) related to VSV Indiana by CF and N. tests was isolated from a pool of Gigatolaelaps mites, combed from 11 Terrestrial Rice Rats (*Oryzomys laticeps velutinus*) caught at the Bush Bush field station of the Trinidad Regional Virus Laboratory, in eastern Trinidad. In 1962 three further isolations of this agent were made, one each from sentinel mice, a rodent and a new species of Culex mosquito.

Serological evidence indicates that the forest floor rodent species in Bush Bush are frequently infected with this agent. In the laboratory, viremia could not be shown to occur regularly in these animals.\(^{11,12}\)

Tr 4-233 isolated in Trinidad and Be Ar 39377 isolated in Belem, Brazil, South America are related to but not identical with the Indiana serotype of vesicular stomatitis (VS) virus. The Tr 40233 isolate has been compared with Indiana type isolates by complement fixation, (1962 Report Rockefeller Foundation Virus Laboratory), serum neutralization (Rockefeller Laboratory and University of Wisconsin), and animal inoculation of the tongue of horses and chickens, and does not produce vesicles in cattle, pigs or rabbits. Vesicles are produced in the guinea pig and ferret following intradermal foot pad inoculation. The Indiana laboratory strain produces vesicles in all of the animals mentioned above by the respective routes of inoculation.

The Tr 40233 virus produced death in adult mice when inoculated subcutaneously whereas the VS Indiana Laboratory strain has not caused death by this route of inoculation.
Serum neutralizing indices of cattle and pony sera collected three weeks after inoculation with four Indiana type isolates of vesicular stomatitis virus are presented in Table I and II. The four isolates are as follows: VS-Lab virus isolates 1925 from cattle in Indiana; VS-SJNM virus isolated 1956 from cattle in San Juan Indian Reservation, New Mexico; VS-BT 78 virus isolated 1960 from Phlebotomus sp. in Bocos del Toro Province, Republic of Panama; VS Tr 40233 virus isolated 1961 from Gigatolaelaps mites in Trinidad.

Apart from individual differences in responsiveness of cattle to vesicular stomatitis virus, several observations can be made. Cattle and horses receiving Tr 40233 virus developed neutralizing antibody to Tr 40233 virus alone. Cattle and horses receiving the other Indiana strain, BT-78, Lab and SJNM, usually developed antibodies that neutralized Tr 40233 virus. The neutralizing indices of the heterologous antisera for Tr 40233 virus, however, were always low when compared to the homologous virus.

**TABLE I**

Serum Neutralizing Indices of Cattle Exposed to Different VSV Isolates

<table>
<thead>
<tr>
<th>Virus Isolates</th>
<th>BT-78</th>
<th>Tr 40233</th>
<th>Indiana</th>
<th>SJNM</th>
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<tr>
<td><strong>Serum Samples</strong></td>
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<td>90*</td>
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</tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
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<td>808</td>
<td>2800</td>
<td>1250</td>
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<tr>
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<td>0</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>SJNM H</td>
<td>250</td>
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<td>250</td>
<td>400</td>
</tr>
</tbody>
</table>

560 180 100 320

* Dilution of serum that neutralized the TCD50 of the respective strains.

**TABLE II**

Serum Neutralizing Indices of Equines Exposed to Different VSV Isolates

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<td>90</td>
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</tr>
<tr>
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</tr>
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Virus TCD50 3200 320 320 100
Foot-and-Mouth Disease Virus (FMDV)

It was reported several years ago by workers at the State Veterinary Research Institute in the Netherlands that a large proportion of cattle which had recovered from FMD may harbor the causative agent in saliva during a period of several months. These workers showed that the virus may be demonstrated in material collected from the esophagus by inoculation into unweaned mice or susceptible cattle. They concluded that there is a continual formation of small quantities of infective materials in cattle carrying FMD virus. After contact with clinical cases, vaccinated animals may develop a similar carrier state without having shown symptoms of the disease. These same workers reported that susceptible oxen kept in contact with such carriers remained unaffected, even if the oral cavity were swabbed with infective saliva. FMD did not occur in vaccinated or unvaccinated cattle or in unvaccinated pigs if such animals were introduced into a herd known to contain carriers or if they were kept on the same premises with such animals. They seem to discount the purely mechanical carrier animal.¹

Research workers at the Animal Viruses Disease Institute at Pirbright, England have studied the effects of various inactivating agents on the virus and the ribonucleic acids, (RNA) of FMDV. In this work FMDV was partially inactivated by several methods: incubation with dilute formaldehyde or acetyleneimine, ultraviolet irradiation (UV), heating or by mixing with type specific antiserum, trypsin, or extracts from cells susceptible to the virus. The serological properties of the treated virus preparations were studied by complement fixation in agar diffusion tests and their content of infective RNA determined by phenol extraction. The ability of the treated preparation to attach to susceptible cultivated pig kidney cells was examined. The decrease in viral infectivity when FMDV was treated with formaldehyde, acetyleneimine, UV or heat at 25°C or 37°C was proportional to the loss of infective RNA with little impairment of its serological properties or ability to attach to susceptible cells. In contrast, loss of viral infectivity on mixing with antiserum, trypsin or cell extracts was due to the failure of the virus to attach to susceptible cells. The viral RNA is still present in infective form in these mixtures.²

Workers at the Research Institute in Great Britain have studied the antigenic differences of various strains of type SAT-1 FMDV. Cattle inoculated with formalized antigen of two strains of the virus develop good immunity to the experimental infection with the identical strain with little resistance to the other strain. In vitro the results of complement-fixation tests and serum virus neutralization tests in tissue culture were consistent with the observations made in vivo. The results of studies on the serological relationship of four strains of SAT-1 were also studied and these workers point out the importance of strain differences in the epizootiology and control of the disease. Their conclusions were essentially that results from use of vaccines under field conditions may be modified considerably by the interval between vaccination and exposure and by a variety of other extraneous factors which may mask the effects of antigenic differences between the vaccine strain and the field strain of virus. Although it seems
that most strains of the virus of FMD can be grouped into distinct types by serological and cross-immunity tests, there is an increasing volume of evidence of wide variation within these type groups. Inasmuch as the serological and cross-immunity studies of the intermediate strains within a type are not complete and marked variations are known to exist among strains, at the moment it would appear best to continue to refer to the strain as of the same type.3

Mackowiak, et al. have studied the duration of immunity conferred in young cattle vaccinated with FMD vaccine. Their work was carried out with animals from three to six and six to nine months of age. It appears from this work that with one vaccination, it is more difficult to obtain a solid and lasting immunity in calves than it is in adult cattle. This is also for re-vaccination and suggests that young animals should have sensitizing vaccination followed by a re-vaccination and sometimes by second re-vaccination. Re-vaccination is advisable for animals six months of age and almost obligatory under three months of age.4

Cunliffe, at the Plum Island Animal Disease Laboratory, studied the antibody response in a group of swine after infection with FMDV. He demonstrated virus-neutralizing antibody in swine for 128 days after infection. The antibody first appeared at three days, rose to peak levels between seven to 10 days and regressed to a plateau by 28 days. After 28 days, there was little change in mean antibody titer. An attempt to re-infect 10 swine at 28 days postinfection was not successful. At 128 days, the immune status of four convalescent swine neutralized more than four logarithms of virus in an in vivo titration. In another group of five convalescent swine, one developed vesicular lesions when exposed to infected swine. Efforts to demonstrate latent virus in one pig 128 days after the infection were not successful.

Van Bekkum et al.6 in the Netherlands studied the immunogenic response following vaccination with types O and A vaccine. In their work, serum neutralization studies against types O and A, FMDV, involved 73 cattle vaccinated with types O, A, C, FMD vaccine and held under field conditions in an area free from FMD for several years. Serum specimens were obtained immediately before vaccination and at intervals of three months or less for 107 weeks following vaccination. Cattle ranged in age from those old enough to have had several annual vaccinations to young animals born in 1957 and vaccinated for the first time in the beginning of the study. Antibody titers of serum samples collected from young animals following primary vaccination declined rapidly. However, most titers of serums from animals vaccinated two or more times remained high throughout the 107 weeks.

Cunha and Honigman7 have compared serum tests in mice for the detection of FMD antibody. Using unweaned mice in a comparison between the serum protection test and the serum neutralization test, it was found that the presence of heterologous virus type antibody was revealed by the serum neutralization test in the sera of cattle during early convalescence but was not revealed by the serum protection test. These different results could be due to greater sensitivity of the serum neutralization test.
or greater specificity of the serum protection test. These workers concluded that the serum protection test was more specific, although less sensitive and that the results generally provided a better indication of the antibody picture in cattle.

Hyde and Graves compared two routes of inoculation of FMDV in guinea pigs. They concluded that the inoculation of the tongue is a more sensitive method for the detection of FMDV than is inoculation of the metatarsal pad. This may be due to the retention of the inoculum in the tongue in which leakage is minimal. The workers explained that this method also offers the advantage of leaving four feet to observe for generalized lesions. It also gives reasonable assurance that the challenge dose of virus has been retained, thus allowing for more quantitative studies.

It has been previously reported that the virus of FMD could be grown in a cell line derived from hamster kidney. Field strains of the virus, as well as vaccine strains modified in pathogenicity for cattle by passage in mice grows well in these cells and high titers are obtained. Mowat et al. have used the baby hamster kidney cell as a culture method for the production of established live modified vaccines and have stated that it offers advantages in terms of simplicity of preparation and eliminates the need for a large supply of unweaned mice. This method also appears to offer a very economical method for the production of live modified FMD vaccines.

Van Bekkum has reported the results of vaccination of pigs in the Netherlands against FMD. During the 1961-62 epizootic, 25,000 pigs on 600 farms in the infected area were inoculated with monolayer Frenkel-type vaccine, as used for cattle. Two inoculations were given one or two weeks apart. Herds on 16 farms found to be infected one to nine days after injection of the first dose were destroyed. The incidence of disease was less in vaccinated than in unvaccinated herds in the same district, but the difference was not significant. Vaccinated pigs have a solid immunity of short duration as shown by challenge inoculation. The practical applicability of the method, therefore, seems limited. This work points out further the need to study FMD in swine.

Work on modified FMD vaccines are continuing in a number of countries. Workers in West Germany have attenuated FMDV in cultures of calf embryo kidney cells. Workers at the Research Institute, Pirbright, England, have used unweaned mice. Workers at the Pan American FMD Center in Rio have used embryonating chicken eggs and young rabbits. Such vaccines are being used in increasing quantities in control field experiments and considerable information on the value of such products should be available within the next few years.

REFERENCES


OFFICERS, CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS FOR 1964

L. W. TURNER  
Chairman

E. P. POPE  
Secretary

J. G. MILLER  
Co-Chairman
Memorandum of Agreement between United States Livestock Sanitary Association and Conference of Veterinary Laboratory Diagnosticians

An Evaluation of Serological Techniques for the Diagnosis of Anaplasmosis and Equine Piroplasmosis, E. I. Pilchard and Miodrag Ristig

Methods of Identification of Certain Etiologic Agents of Atrophic Rhinitis, R. F. Ross

Diagnosis of Coccidiosis of Cattle and Sheep by Histochemical and other Techniques, L. R. Davis and G. W. Bowman

Helminths of Ruminants: Geographic Distribution and Economic Importance, Willard W. Becklund

Differential Diagnosis of Insecticide Poisoning in Livestock, R. D. Radeleff

Instrumentation for Scientific Excellence in Veterinary Medical Laboratories, V. B. Robinson


The Diagnosis of Scrapie, M. A. McDaniel and L. G. Morehouse

Laboratory Consultation and Evaluation of Routine Laboratory Studies on Swine Diseases, F. E. Mitchell and V. B. Robinson

Antibody Response of Turkeys Exposed to Mycoplasma Gallisepticum, R. Yammamoto, W. E. Babcock and E. M. Dickinson

Arthropod Vectors of Some Livestock and Poultry Diseases, D. W. Anthony


MEMORANDUM OF AGREEMENT

Memorandum of Agreement for the Affiliation of the Conference of Veterinary Laboratory Diagnosticians with the United States Livestock Sanitary Association. It is mutually agreed that:

1. All C.V.L.D. members attending the annual meetings of the two organizations shall be registered as U.S.L.S.A. registrants and charged the established U.S.L.S.A. registration fee.

2. All registration fees collected shall be retained by the U.S.L.S.A. except for $200.00 or any lesser amount as agreed to by both parties, which will be annually paid to the C.V.L.D. treasury.

3. The U.S.L.S.A. will publish the annual proceedings of the C.V.L.D. in the official proceedings of its annual meetings.

4. Any C.V.L.D. members who are not U.S.L.S.A. members shall have the opportunity of purchasing the U.S.L.S.A. proceedings at the same cost as that of an individual membership in the U.S.L.S.A.

5. The Executive Secretary of the U.S.L.S.A. shall provide acceptable meeting facilities for C.V.L.D. annual meetings.

6. This memorandum of agreement may be terminated upon one year's notice by either of the above two parties.

Signed

U.S.L.S.A. President

C.V.L.D. Chairman

Approved by the Executive Committee of the United States Livestock Sanitary Association 5:40 p.m., Thursday, October 17, 1963, in Executive Session at the 67th Annual Meeting of Western Skies Hotel, Albuquerque, New Mexico.
AN EVALUATION OF SEROLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF ANAPLASMOSIS AND EQUINE PIROPLASMOSIS

E. I. Pilchard* and Miodrag Ristic**

Urbana, Illinois

INTRODUCTION

Anaplasmosis of cattle, caused by the rickettsia-like organism *Anaplasma marginale* and equine piroplasmosis, caused by the protozoa *Babesia caballi* and *Babesia equi* (Berger’s Manual, 1957; Levine, 1961), are both hemotropic, have certain similarities in mode of transmission by insect vectors, and produce similar clinical syndromes in their respective natural hosts. Cattle recovered from acute anaplasmosis and horses recovered from acute piroplasmosis continue to carry, apparently, the causative organism for many months or years. These carrier animals serve as important sources of infection for susceptible stock. Effective control and ultimate eradication of both diseases awaits practical and efficient detection of carrier animals, control of insect vectors and identification of carriers among the fauna of ecosystems of which the domestic animals at risk are a part.

Anaplasmosis and equine piroplasmosis are conveniently diagnosed by recognition of morphologic and tinctorial characteristics of the respective microorganisms in the erythrocytes of acutely infected animals. However, during the carrier phase of infection only a few or no organisms may be found, making diagnosis uncertain. Thus, serologic methods are needed for detection of carrier animals.

The purpose of this paper is to discuss serologic techniques for the diagnosis of bovine anaplasmosis and equine piroplasmosis, to report the results of recent investigations at our laboratories*** and to evaluate the relative usefulness in a control program of means now available for the detection of carrier animals.

**Diagnosis of anaplasmosis in cattle.** Diagnosis of anaplasmosis by examination of blood films stained with various histochemical stains for the

This investigation was supported in part by Research Grant AI-03315, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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presence of marginal Anaplasma bodies in the erythrocytes usually becomes uncertain 16 to 26 days following their appearance (Price et al., 1954). Acridine orange (AO) staining (Gainer, 1961) increases the period of time during which visualization is useful, but this technique has the disadvantage of staining nucleic acid of immature erythrocytes (i.e., Howell-Jolly bodies) which may resemble marginal Anaplasma bodies. A comparison between the complement-fixation (CF) and the AO test revealed no conclusive evidence concerning the value of the latter test (Gainer, 1961).

Complement-fixation (CF) test for anaplasmosis. Complement-fixing Anaplasma antibodies are detectable in infected cattle beginning at 10 to 25 days following experimental inoculation (Schoening, 1953; Ristic and Mann, 1963). The CF test has been reported to be 96 percent accurate in detecting carriers (Price et al., 1954), 97.9 percent accurate in detecting known positive cattle (Gates et al., 1954a), and 91.8 percent accurate in differentiating normal, acutely infected and carrier cattle (Gates et al., 1954b). These workers presented no data to indicate the chronological frequency distribution of sample collection. That CF antibody may persist for long periods of time is suggested by the report by Dykstra et al., (1948) in which an Anaplasma carrier became CF-negative after 12 years.

The presence in a bovine of inapparent Anaplasma infection is usually determined experimentally by the development of the acute disease in a splenectomized calf which has been injected with a sample of blood from the suspected carrier. Schoening (1953) inoculated nine splenectomized calves respectively with blood from nine CF-positive cattle. Seven of the nine recipients (78 percent) developed anaplasmosis. Four conclusions might be made from these findings: 1) calf-inoculation is not a reliable means of detecting carrier animals; 2) false-positive results were obtained with the CF technique in two of nine animals; 3) CF antibody persists following the disappearance of Anaplasma from certain individual animals; or 4) infective Anaplasma might not always be present in sufficient concentration to produce an infection in a susceptible recipient host. The duration of the period during which the CF test can detect anaplasmosis in individual cattle is not known with certainty.

The specificity of the CF test is dependent upon the purity of the antigen preparation which is employed. The antigen currently prepared by the USDA appears to be satisfactory (Gates, 1953; Price et al., 1952; Gates et al., 1954a). Even so, newer methods aimed at the production of an antigen suspension devoid of antigenic determinants other than those of Anaplasma should be sought. Cross-reactions between Anaplasma and bovine Eperythrozoon have been reported (Kreier and Ristic, 1963).

A small percentage of bovine serums contain substances which bind complement nonspecifically (i.e., anti-complementary) and therefore cannot be CF tested. Also, testing of serums by the CF method at dilutions of less than 1:5 is considered impractical due in part to occurrences of false-positive reactions. The CF tests is relatively cumbersome and expensive to perform. In addition to serum and antigen three other different biologically variable components are required for the performance of the CF test. Hemolysis may render serum samples unsuitable for the CF test.
Inherent variability, cost and the difficulties in performing the complex CF test, justify vigorous search for simpler diagnostic methods.

Capillary-tube agglutination (CA) test for anaplasmosis. Anaplasma agglutinating antibodies can be detected by means of the CA test developed by Ristic (1962). In the majority of cases, the earliest CA reactions were observed before the peak of acute infection. At the time the first agglutinating antibodies appeared, Giemsa-stained blood smears usually revealed marginal Anaplasma bodies in three to six percent of the erythrocytes (Ristic, 1962). In a study where small inoculum doses were used (Ristic and Mann, 1963) the CA test was positive beginning 30 days following inoculation or five days after the first positive CF reactions.

To determine the specificity of the CA test, serums against the most common cattle pathogens were tested by this technique. The CA antigen did not cross-react with serums containing antibodies specific to Leptospira pomona, Pasteurella multocida, Corynebacterium pyogenes, Clostridium chauvoei, Clostridium septicum, Vibrio fetus, Brucella abortus, Staphylococcus agalactiae, virus diarrhea agent, infectious bovine rhinotracheitis virus, and Eperythrozoon (Welter and Zuschek, 1962).

The CA test is simple and economical to perform. The test can be performed in the field and information regarding the status of an animal can be obtained without delay. Serums are inactivated for 30 minutes at 56°C and tested undiluted or following serial dilution. Reagents are the serum to be tested and the standardized test antigen. The CA antigen was found to be a cell free suspension of the initial bodies which are known to occur singly or as subunits of the marginal Anaplasma bodies in infected erythrocytes (Ristic and Kreier, 1963). Substances which might interfere with the performance of the CA test have not been encountered in bovine serums. However, 0.5 percent phenol added as a preservative to the serum before heat-inactivation decreases the sensitivity of the CA reaction. A final serum concentration of merthiolate 1:10,000 or phenol 0.25 percent, added after inactivation of serum by heat are found to be satisfactory preservatives when needed.

Results of CA and CF tests in Illinois. Data obtained from the testing of 4,044 bovine serums for anaplasmosis at the Illinois State Diagnostic and Research Laboratory* during the period of 18 months ending on August 26, 1963, are presented in Table I. In the absence of other correlative data, such as the results of direct examination of blood smears, subinoculation of splenectomized calves, or clinical histories of the animals tested, conclusions relative to the validity of test results cannot be made. That the CF test may be slightly more sensitive than the CA test in revealing more positive reactions is suggested but not established by negative CA test results on four CF-positive serums. However, the finding that of 75 serums submitted to the CA and CF tests 71 serums were positive to both tests, suggests little difference in sensitivity. This conclusion is in

*The Diagnostic and Research Laboratory is operated by the Illinois Division of Livestock Industry in cooperation with the College of Veterinary Medicine, University of Illinois, Urbana, Illinois.
TABLE I

Results of the Capillary-Tube Agglutination (CA) and Complement-Fixation (CF) Test of Bovine Serums Received at the Illinois State Diagnostic and Research Laboratory During the Period of 18 Months Ending on August 26, 1963

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total serums tested</td>
<td>4,044</td>
</tr>
<tr>
<td>Total serums CA-positive</td>
<td>1,131</td>
</tr>
<tr>
<td>Subtotal of serums both CF- and CA-tested</td>
<td>75</td>
</tr>
<tr>
<td>Serums CF- and CA-positive</td>
<td>71</td>
</tr>
<tr>
<td>Serums CA-positive and CF-negative</td>
<td>1</td>
</tr>
<tr>
<td>Serums CF-positive and CA-negative</td>
<td>4</td>
</tr>
</tbody>
</table>

agreement with a recent study of Kuttler (1963). Segregation of CA-positive cattle in several herds in southern Illinois was successful in controlling anaplasmosis (Ristic, 1963).

In order to ascertain the accuracy with which the CA and the CF tests may detect Anaplasma-free cattle, 200 serum samples from a northern Illinois dairy herd, which had no history of anaplasmosis, were examined by the CA and CF tests (Table II). Positive with the CF test were: six animals at 4+, seven animals at 3+, eight animals at 2+, and 12 animals at 1+. In the CA test one animal reacted as suspect. Subinoculations of 500 ml. of blood from the six animals reacting strongly (4+) in the CF test and one animal reacting as suspect in the CA test were made into splenectomized calves (Table III). None of the calves showed hematologic evidence of anaplasmosis and all remained negative when examined by the CA test. Cross-reactions with Eperythrozoon bovis were encountered with the CF test in recipient calves at two and four weeks following inoculation but not at six, 10 and 12 weeks. These results indicate that the CA test is an accurate technique for classifying uninfected cattle as such.

The number of false-positive (4+) CF test reactions substantiated by the results of subinoculation, agrees closely with the data recently reported by Merriman et al., (1962). The remaining suspect reactions (1+ to 3+) could be due to the presence of naturally occurring complement binding antibodies in bovine serums or to denaturation of the serum due to its exposure to heat following treatment with phenol. The latter point is substantiated in the studies of Heck et al., (1962). These workers stated: "It could be concluded, subject to inoculation studies, that the indicated CF

TABLE II

Results of Examination of a Herd of 200 Anaplasmosis-Free Cattle by the Complement-Fixation (CF) and the Capillary-Tube Agglutination (CA) Tests

<table>
<thead>
<tr>
<th>Tests Used and No. of Reactors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>CA</td>
<td>CF</td>
</tr>
<tr>
<td>Suspect Positive</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>2+</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>3+</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4+</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

| Totals                         |       |       |
| CA                            | CA    | CF    |
| Suspect Positive               | 0.5%  | 0     |
| 15.5%  | 3%    | 3%    |
TABLE III
Results of Subinoculation of Blood from the CF-Positive (4+) and the CA-Suspect Animals into Splenectomized Calves

<table>
<thead>
<tr>
<th>Test</th>
<th>Donor Cow</th>
<th>Splenectomized Calf</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF (4+)</td>
<td>25</td>
<td>1</td>
<td>Epy.*</td>
<td>N**</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>2</td>
<td>Epy.</td>
<td>Epy.</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>3</td>
<td>N</td>
<td>Epy.</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>4</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>5</td>
<td>Epy.</td>
<td>Epy.</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>6</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>CA (Suspect)</td>
<td>105</td>
<td>7</td>
<td>Epy.</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*Eperythrozoon bovis were observed in Giemsa-stained blood film, Anaplasma CF test result was 4+ and Anaplasma CA test result was negative.
** = No hemoparasites were observed, Anaplasma CF test result was either suspicious or negative and Anaplasma CA test result was negative.

results from phenolized serum may not be true indication of the presence of homologous antibodies."

*Fluorescence antibody (FA) and acridine orange (AO) tests for anaplasmosis.* The use of the direct and indirect FA-technique (Ristic et al., 1957; Ristic and White, 1960) has permitted the detection of marginal Anaplasma bodies and its subunits "initial bodies" (Ristic, 1962).

The FA test is relatively simple to perform, but requires an ultraviolet microscope and other equipment not available at all laboratories. Nonspecific fluorescence must be subjectively evaluated by the person performing the test. The possibility of using the FA test to detect carrier animals has not been explored. Difficulty encountered in differentiating initial Anaplasma bodies from minute foci of nonspecific fluorescence limits the use of the FA test in its present form to research.

*Other tests for anaplasmosis.* Anaplasmosis has been diagnosed by means of the gel-diffusion technique and by indirect hemagglutination (Ristic et al., 1963a; Ristic and Mann, 1963). The gel-diffusion method permitted demonstration of Anaplasma precipitating antibodies at 35 and 41 days following experimental inoculation of two calves (Ristic and Mann, 1963). Precipitins appeared later than CF or agglutinating antibody (Ristic and Mann, 1963).

Precipitation of euglobulin from serum of Anaplasma carriers was reported by Boynton and Woods (1935) but owing to nonspecificity, this technique is not considered useful as a diagnostic test. A skin test (Ristic and Mann, 1963) and Fulton's reaction (Rossi et al., 1956) are mentioned as methods which were found unsuccessful for the diagnosis of anaplasmosis.
Tests for equine piroplasmosis. The laboratory diagnosis of equine piroplasmosis has been accomplished up to the present time by demonstrating the mature or dividing forms of Babesia caballi (Piroplasma caballi) or Babesia equi (Nuttallia equi, Piroplasma equi) in the erythrocytes of infected horses, mules or donkeys. Babesia equi has been found also in the zebra. At the height of acute piroplasmosis, about four percent of the erythrocytes contain parasites recognizable by Giemsa staining. The number of parasites decreases rapidly as the infection progresses. At the 31st day following experimental inoculation of a horse, Ristic et al., (1963b) found that 0.1 percent of the erythrocytes contained Babesia. Agglutinating antibodies were present in the serum at this time.

Sippel et al., (1962) found the acridine orange (AO) stain useful in differentiating Babesia from the Howell-Jolly bodies which appear during the anemia which accompanies piroplasmosis in the horse. Howell-Jolly bodies reportedly do not contain deoxyribonucleic acid (DNA). DNA causes Babesia to fluoresce yellow-green with acridine orange stain.

Fluorescent antibody (FA) test for equine piroplasmosis. The FA test, employing serum from a horse infected with Babesia (Florida isolate), has been used by Ristic et al., (1963b) to demonstrate the organism in the erythrocytes of infected horses. Fluorescein-labelled anti-Babesia antibody did not stain bovine Anaplasma, and fluorescein-labelled anti-Anaplasma antibody did not stain equine Babesia (Ristic et al., 1963b). A one-step fluorescein-labelled antibody inhibition test specifically inhibited the FA reaction for equine piroplasmosis (Ristic et al., 1963c).

The possible usefulness of AO and FA staining techniques in the recognition of carrier animals has not been assessed.

Serologic tests for equine piroplasmosis. Using a technique similar to that employed for preparation of Anaplasma CA antigen, Ristic et al., (1963c) have prepared a CA antigen from Babesia-containing blood, and used it for the demonstration of agglutinating antibodies in sera of infected horses. Agglutinating antibodies were also detected in 19 of 20 known positive horses from Florida and Georgia. However, the Piroplasma CA antigen was found highly perishable; and, therefore is of no practical value in its present form.

More recently, Ristic and his associates (1963c) prepared a precipitinogen from Piroplasma-infected erythrocytes and employed it in a gel precipitation (GP) test for detection of an antibody present in the sera of acutely infected and carrier horses. This antigen proved more stable than the CA antigen and the GP test was accurate in experimental trials. Serums from 17 known infected horses were accurately diagnosed by the GP technique. In addition, 11 known infected horses were tested by the GP test with equal accuracy at Doctor Sippel's laboratory in Florida (Sippel and Gainer, 1963). Only sharply defined straight precipitation lines between the antigen and the serum wells are considered the product of a specific antigen-antibody reaction in the GP test for equine piroplasmosis.
DISCUSSION

The limited information which is now available on the immunology of anaplasmosis and equine piroplasmosis makes difficult a direct comparison of the serologic techniques for diagnosis of the two diseases. Both the CF and the CA tests appear to be useful in the diagnosis of anaplasmosis in cattle in the acute and carrier phases of infection. In their present forms, the gel diffusion, indirect hemagglutination and FA tests appear to be primarily applicable to research techniques.

The diagnosis of acute anaplasmosis by the CF test is frequently possible one to three days before agglutinating antibody in serum from the same animal can be detected by the CA test. Both tests react positively to antibodies which first appear during the phase of infection in which marginal bodies are easily identified in blood smears. The CF and CA tests are both capable of detecting cattle inapparently infected with Anaplasma.

Both the CA and CF tests may be used to perform end-point titrations of anaplasmosis antibody. Both tests require a comparable period of time to react, following the final step of the test procedure preceding reading. Final reading of the CA test is made at 24 hours, although agglutination may be observed at five to 10 minutes with serums of high antibody titer.

The total effort necessary for the performance of the CF test is considerably greater than that required for the CA test. Preliminary heat-inactivation of serums is required for both tests. Preliminary titration of complement and hemolysin (amboceptor), preparation of washed sheep erythrocyte suspensions and control tests for anti-complementary activity required in the CF procedure are obviated by the CA test. For the CA test, serum and then antigen is introduced into a disposable capillary tube; the tube is then set vertically in a bit of plasticine until read. Willers (1962) reported that one technician can perform an average of 360 CF tests during one eight-hour working day, with a maximum of 597 tests per day. In contrast with these figures, one technician using the CA test can screen approximately 800 undiluted, heat-inactivated serums in an eight-hour working day.

Upon reviewing the procedures used for preparation of the CF (Gates, 1953) and CA (Ristic, 1962) antigens, the cost of production of each is probably comparable. All CF antigen used in the United States is produced by the United States Department of Agriculture at an estimated cost of two to five cents per test (Gates et al., 1954b). Additional reagents and equipment are required for the performance of the CF test. CA test antigen is available commercially* at a retail cost of about 14 cents per test with equipment for the performance of the test included. One Anatest kit costs $18.17 and is sufficient for the performance of approximately 130 tests (Pilchard, 1963).

In an effort to develop methods for control of anaplasmosis the Anaplasmosis Committee of the United States Livestock Sanitary Association has repeatedly emphasized the need for development of a simple test for

diagnosis of the disease. The CA test meets this and other objectives of a dependable serologic test. The test is simple, economical, accurate and specific in identification of *Anaplasma* positive and negative animals (Ristic, 1962; Welter and Zuschek, 1962; Ristic, 1963). The CA test was found to be of comparable sensitivity to the CF test (Kuttler, 1963). In order to make evaluation of the CA test possible on a broader scale the CA antigen should be made available free of cost, as in the case with the CF antigen, to state diagnostic laboratories and other state and federal agencies.

The gel-diffusion test for anaplasmosis (Ristic, Mann and Kodras, 1963a) appears useful primarily as a research technique since antibody concentrations sufficient to be detected by this technique apparently do not persist in all carrier animals. The indirect HA test (Ristic and Mann, 1963) appears to have no advantage over the CA test for anaplasmosis (Ristic and Mann, 1963).

At the present time, the direct examination of blood smears for the presence of *Anaplasma* bodies, together with the CF or CA test appear to be sufficient for the diagnosis of anaplasmosis in cattle.

In the case of equine piroplasmosis, the encouraging results of the GP test obtained by Ristic *et al.*, (1963c) may be taken to suggest this is the only useful serologic test now available for the diagnosis of this disease. Equine piroplasmosis is usually diagnosed by recognition of the causative organism in the erythrocytes of typically affected animals. The acridine orange stain and the FA technique can facilitate visualization of the small numbers of *Babesia* in carrier animals. The GP test should be a valuable diagnostic aid in current efforts to control equine piroplasmosis in the United States.

REFERENCES


ANAPLASMOSIS AND EQUINE PIROPLASMOSIS


METHODS OF IDENTIFICATION OF CERTAIN ETIOLOGIC AGENTS OF ATROPHIC RHINITIS

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Ames, Iowa

The concept that several different agents will cause atrophic rhinitis has evolved during the past seven years.\textsuperscript{1,2,3} \textit{Bordetella bronchiseptica}, \textit{Pasteurella multocida}, \textit{Hemophilus suis}, the inclusion body rhinitis virus and a large filter passing agent are incriminated as causes of rhinitis and turbinate atrophy in swine.\textsuperscript{1,2,3,4,5,6,7} Our knowledge regarding the pathogenic natures, economic effects and distribution of these agents is rapidly increasing. Nevertheless, many questions are unanswered. Additional unrecognized agents capable of causing upper respiratory tract disorders in pigs may exist.

In a recent sampling nasal mucus was collected from four eight-to-10 week-old purebred swine from each of 87 of the more progressive Iowa swine herds.\textsuperscript{5} Examination of these nasal specimens revealed that 54 percent of the herds had \textit{Bordetella bronchiseptica}, 48 percent had \textit{Hemophilus suis} and five percent had \textit{Pasteurella multocida}. In another survey, samples of nasal mucus were collected from pigs submitted for various reasons to the Iowa Veterinary Diagnostic Laboratory. Again 87 herds were sampled. Cases from each herd consisted of one to three pigs. The ages of the pigs ranged from one week to four months. Thirty eight percent (33/87) had \textit{Bordetella bronchiseptica}, 23 percent (20/87) had \textit{Hemophilus suis} and nine percent (8/87) had \textit{Pasteurella multocida}. In 28 percent (24/87) of these herds gross turbinate atrophy was present in 25 percent (5/20) of the herds with \textit{Hemophilus suis}, 24 percent (8/33) of the herds with \textit{Bordetella bronchiseptica} and 38 percent (3/8) of the herds with \textit{Pasteurella multocida}.

Nasal mucus is collected by veterinary practitioners from suspect herds and submitted to the Iowa Veterinary Diagnostic Laboratory. During July, August and September, 1963, 32 cases of this type were submitted. Fifty six percent (18/32) of the herds had \textit{Bordetella bronchiseptica}. \textit{Hemophilus suis} was detected in 16 percent (5/32) of the herds. \textit{Pasteurella multocida} was not detected.

Additional cases consisting of one or two live pigs of various ages were submitted to the Iowa Veterinary Diagnostic Laboratory specifically for postmortem and bacteriologic examination of the nasal cavity. Many of these animals came from herds with clinical rhinitis. Again cases consisted of from one to three pigs. Of 28 cases submitted for this purpose

Appreciation is expressed to Dr. W. P. Switzer, Dr. P. C. Bennett, Dr. V. A. Seaton, Dr. J. R. Andersen and Dr. G. S. Firkins for their helpful suggestions and for collection and processing of some of the specimens.
in July, August and September, 1963, 68 percent (19/28) had *Bordetella bronchiseptica*, 11 percent (3/28) had *Hemophilus suis* and 11 percent (3/28) *Pasteurella multocida*. Gross turbinate atrophy was detected in 71 percent (20/28) of the cases. In 68 percent (13/19) of the cases with *Bordetella bronchiseptica* gross turbinate atrophy was detected.

Incidence of Three Bacteria in the Nasal Cavities of Iowa Swine

<table>
<thead>
<tr>
<th></th>
<th>87 herds</th>
<th>87 herds</th>
<th>32 herds</th>
<th>28 herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td><em>Bordetella bronchiseptica</em></td>
<td>47</td>
<td>54</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td><em>Hemophilus suis</em></td>
<td>42</td>
<td>48</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

* Eighty seven herds - one to three live pigs submitted to Iowa Veterinary Diagnostic Laboratory for various reasons.
**Thirty two herds - three to fifty samples of nasal mucus submitted to Iowa Veterinary Diagnostic Laboratory.
***Twenty eight herds - one to three live pigs submitted to Iowa Veterinary Diagnostic Laboratory for examination of the nasal cavity.

*Bordetella bronchiseptica* causes a characteristic pneumonia in young pigs.\(^8\)\(^9\) It can usually be isolated from the turbinates as well as the pneumonic lungs of the affected pig. A significant observation is that *Bordetella bronchiseptica* colonizes on the tracheal mucosa of experimentally infected pigs.\(^6\) This has led to the finding that the organism will frequently colonize on the tracheal mucosa in young pigs in the field and cause coughing with no gross evidence of changes in the trachea other than a slight increase in mucus. In some of these cases no gross pneumonic lesions are observed.

Relatively simple techniques are available for the detection of *Bordetella bronchiseptica*, *Hemophilus suis* or *Pasteurella multocida* and the inclusion bodies of inclusion body rhinitis. A specific etiologic diagnosis is rendered when one of these agents is demonstrated in pigs with symptoms and postmortem evidence of rhinitis. Detection of any of these agents in pigs without clinical or postmortem evidence of rhinitis indicates a potential for respiratory disease in the herd. However some isolates of *Pasteurella multocida*\(^1\)\(^1\) and *Hemophilus suis*\(^1\)\(^1\) have been found to be non-pathogenic when experimental transmission trials were conducted.

For detection of *Bordetella bronchiseptica*, *Hemophilus suis* or *Pasteurella multocida* nasal mucus is collected from several live pigs on the farm or at necropsy in the laboratory. The optimum time for collection is during the early stages of the disease as soon as sneezing is prevalent in the herd. In older pigs turbinate atrophy may progress to the point that extensive secondary invasion will prevent detection of the primary agent. The number of samples will depend on the herd size and the extent of sneezing. In general five to fifteen sneezing pigs should be sampled.
One may detect *Bordetella bronchiseptica*, *Pasteurella multocida* or *Hemophilus suis* in the nasal cavities of breeding stock by use of these techniques. It is important that each sow and boar be sampled since a relatively low percentage may be carriers of *Bordetella bronchiseptica*. Our knowledge about the adult carriers of *Hemophilus suis* and *Pasteurella multocida* is incomplete at the present time. However it seems reasonable to assume that a complete herd check of adult stock would reveal their presence as well as the presence of *Bordetella bronchiseptica*.

One external naris of a restrained pig is carefully cleaned with cotton moistened with 70 percent alcohol. The cotton is twisted to a conical shape to facilitate cleaning of the entire external naris. This step is repeated two to three times. After a brief pause to allow drying of the alcohol, a sterile cotton tipped applicator is carefully inserted about one-third of the length of the nasal cavity using a gentle twisting motion. Each sample is placed in a sterile tube for transport to the laboratory. It is advisable to refrigerate the samples to minimize drying. The samples should be streaked on bacteriologic media within four hours after collection.

The nasal mucus is streaked on five percent horse or bovine blood agar and MacConkey's agar* fortified with one percent dextrose. A staphyloccoccus known to support the growth of *Hemophilus suis* is streaked once across the center of the blood agar plate. Both plates are incubated at 37 degrees Centigrade.

After incubation for 24 hours the blood agar plate is observed for satellite colonies of *Hemophilus suis* in close proximity to the growth of the staphyloccoccus "nurse" colony. *Hemophilus suis* colonies are minute and semitranslucent. Confirmation of the satellite character of the Gram negative organism on a second subculture is considered diagnostic of *Hemophilus suis*. *Hemophilus suis* will not grow on MacConkey's agar nor will it grow on blood blood agar without the support of the staphyloccoccus.

After 48 hours of incubation colonies of *Bordetella bronchiseptica* are two to three mm. in diameter and are a translucent tan color with a dark center on MacConkey's agar. The organism grows well on blood agar, however, the MacConkey's agar is quite selective for *Bordetella bronchiseptica* and facilitates its recognition.

Suspect colonies of *Bordetella bronchiseptica* are selected and inoculated into an infusion or tryptose broth. After incubation at 37 degrees Centigrade for 24 hours the broth culture is inoculated into dextrose, litmus milk, urea and citrate. Hydrolysis of urea always occurs within 12 to 24 hours of incubation. Citrate is utilized within one to two days. Litmus milk and dextrose are alkalinized slowly. It is motile and Gram negative.

Routine isolation methods are employed for *Pasteurella multocida*. The organism grows well on blood agar. Typical colonies are selected and incubated in broth. It is Gram negative, H₂S negative, lactose negative, dextrose positive and indole positive.

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*Difco Laboratories, Detroit 1, Michigan*
Inclusion body rhinitis is diagnosed by histopathologic examination of the ventral turbinates. Suspect pigs in the early stages of infection are euthanized, the head is split longitudinally and the ventral turbinates are removed. They are fixed in 10 percent formalin. Decalcification is achieved by adding three parts per 100 of glacial acetic acid to the formalin. Cross sections taken from the anterior one-third of the ventral turbinates are processed in the routine manner. Haemotoxylin and eosin stains satisfactorily demonstrate typical intranuclear inclusions in the cells of the collecting ducts and alveolar cells of the tubuloalveolar glands.

In summary, surveys indicate that *Bordetella bronchiseptica*, *Hemophilus suis* and *Pasteurella multocida* are quite common in Iowa swine. Relatively simple and readily applicable techniques are available for the detection of these organisms in a herd of swine. Information thus obtained may be used to render a specific diagnosis or to give advice concerning the presence or absence of potential pathogens.

**REFERENCES**

The diagnosis of coccidiosis in cattle and sheep is very difficult when only the endogenous stages are available. Sometimes animals may die before the appearance of oocysts in the feces, and the stages in the digestive tract at post-mortem are the only forms that can be used for study and differentiation.

This paper presents some of the methods used in research on coccidia of cattle and sheep at the Regional Animal Disease Laboratory.

MORPHOLOGICAL CHARACTERISTICS

Structural details and locations of the various endogenous stages are particularly helpful in attempting to differentiate the species of coccidia in smears and in sections of intestinal tissues. The following outlines (Tables I, II) may prove helpful. These data are based on results obtained from inoculations of animals used in life history studies, not on natural infections that are commonly complicated by intercurrent infections that are usually impossible to interpret.

In the authors' opinion, the location of the various stages throughout the length of the intestines is not too important in differential diagnoses because too many factors may influence the place of penetration. Hammond et al. found that a partial stenosis caused by surgery resulted in the location of more parasites just above the obstruction as compared with the region below. Davis and Bowman found oocysts in cellophane bags enclosed in perforated gelatine or aluminum capsules intact in the rumen two and three days, respectively, after calves swallowed them. Also, they mentioned reverse peristalsis as the possible reason that some of the endogenous stages are found anteriorly in the digestive tract.

Sporozoites are not listed as it is nearly impossible to use these at this time to diagnose the species in tissues. If oocysts are present, they can usually be identified by their morphology, size, and presence or absence of a polar cap.

ULTRAVIOLET LIGHT WITH FLUORESCING POWDER OR STAINS

Attempts were made to develop a method for tracing small numbers of inoculated oocysts in the digestive tract or in the feces of calves, lambs, and rats. In an effort to preclude the use of expensive radioactive isotopes and special equipment, some trials were made with pigments.
TABLE I
Schizonts and Gametocytes of Coccidia in Sheep, Based on Animal Inoculations in Life History Studies

<table>
<thead>
<tr>
<th>Species and Stages</th>
<th>Maximum size (μ)</th>
<th>Area</th>
<th>Location</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria ahsata⁷</td>
<td>Schizonts 265</td>
<td>Small intestine</td>
<td>Deep in mucosa, sparse in villi</td>
<td>Thick host wall with radiating fibers</td>
</tr>
<tr>
<td></td>
<td>Gametocytes 45</td>
<td>Small intestine</td>
<td>Epithelial cells of glands, sparse in villi</td>
<td></td>
</tr>
<tr>
<td>Eimeria arloingi¹⁸</td>
<td>Schizonts ca. 146</td>
<td>Small intestine</td>
<td>Endothelial cells in villi</td>
<td>Probably millions of merozoites in each.</td>
</tr>
<tr>
<td></td>
<td>Gametocytes -</td>
<td>Small intestine</td>
<td>Epithelial cells</td>
<td>Enlarged villi in clusters</td>
</tr>
<tr>
<td>Eimeria faurei¹⁹</td>
<td>Schizonts ca. 100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eimeria ninakohlyakimovae²⁰</td>
<td>Schizonts ca. 300</td>
<td>Ileum</td>
<td>Epithelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gametocytes -</td>
<td>Ileum, cecum, upper small intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eimeria parva¹⁶,¹⁷</td>
<td>Confusing, mixed infections</td>
<td>Schizonts 256</td>
<td>Throughout small intestine</td>
<td>Mucosa, may be deeply imbedded</td>
</tr>
<tr>
<td></td>
<td>Gametocytes 16</td>
<td>Mostly cecum and colon</td>
<td>Small intestine</td>
<td>Epithelial cells</td>
</tr>
</tbody>
</table>

(Switzer Bros., Cleveland, Ohio) of extremely brilliant hues in daylight and even brighter colors under ultraviolet light. These pigments consisted of powders in small particles (4.5 μ av. size) reported to be relatively harmless even when fed in greater amounts than the trace quantities needed for the study. The powder was mixed two to one with bentonite as a carrier. It was found that when as little as three g mixed with oocysts was given orally to a yearling calf the inoculum could be located in the digestive tract by use of a hand-held Wood's light (UV). The oocysts and
### TABLE II
Schizonts and Gametocytes of Coccidia in Cattle, Based on Animal Inoculations in Life History Studies

<table>
<thead>
<tr>
<th>Species and Stages</th>
<th>Maximum size (μ)</th>
<th>Area</th>
<th>Location</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria alabamensis&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>17.5</td>
<td>Ileum</td>
<td>Epithelial cells</td>
<td>Intranuclear</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>25.2</td>
<td>Mostly ileum. Cecum, upper colon in heavy infections</td>
<td>Epithelial cells</td>
<td>Intranuclear, up to 5 in one nucleus</td>
</tr>
<tr>
<td>Eimeria auburnensis&lt;sup&gt;4,13&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>250</td>
<td>Lower small intestine</td>
<td>Between crypts</td>
<td>Deeply imbedded</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>287.5</td>
<td>Lower small intestine</td>
<td>Mostly near muscularis mucosae</td>
<td>Macroscopic</td>
</tr>
<tr>
<td>Microgametocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrogametocytes</td>
<td>17.9</td>
<td>Lower small intestine</td>
<td>Subepithelial</td>
<td>Mesodermal cells</td>
</tr>
<tr>
<td>Eimeria bovis&lt;sup&gt;12&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>435.2</td>
<td>Lower small intestine, rare in cecum and colon</td>
<td>Endothelium, in lacteals</td>
<td>Macroscopic. 120,000 merozoites (Av.)</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>-</td>
<td>Cecum and colon, rare in ileum</td>
<td>Cytoplasm of epithelial cells of glands</td>
<td>Rare in submucosa</td>
</tr>
<tr>
<td>Eimeria ellipsoidalis&lt;sup&gt;14&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Merozoites in scrapings from middle small intestine</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>-</td>
<td>Mostly ileum, some in posterior 2/3ds of small intestine</td>
<td>Epithelial cells in base of crypts</td>
<td></td>
</tr>
<tr>
<td>Eimeria zurnii&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>16.8</td>
<td>Small intestine. Rare in cecum and colon</td>
<td>Cytoplasm of epithelial cells</td>
<td>Up to 36 merozoites</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>20</td>
<td>Colon and rectum</td>
<td>Cytoplasm of epithelial cells</td>
<td></td>
</tr>
<tr>
<td>Microgametocyte</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrogametocyte</td>
<td>14.5</td>
<td>Colon and rectum. Rare in small intestine</td>
<td>Cytoplasm of epithelial cells</td>
<td></td>
</tr>
</tbody>
</table>
early stages were found adjacent to the brilliantly glowing pigment particles. This system was far better than the old one using carbon particles and microscopy to locate an inoculum.

The fluorescing particles in the feces made it possible to determine for as long as three days which feces on the ground in the feedlot came from inoculated animals. It was suggested that the inert pigments could be used to determine sites of injection of organisms, drugs, chemicals and other substances.\textsuperscript{8}

Another use for ultraviolet light was with fluorescence staining of coccidial oocysts of cattle and of sheep.\textsuperscript{9} Staining of oocysts is extremely difficult. It was found that removal of the outer wall of the oocyst\textsuperscript{22} with antiformin allowed greater penetration of the fluorescent stains such as Acridine Orange, Auramine O, Acridine Yellow, Neutral Red, Primuline Yellow, and Rivanol. Acridine Orange produced more vivid results than did the others. It was used at pH 4.7, or unbuffered, at a concentration of 0.2 percent. Heat-killed and live oocysts were differentiated, to some extent, when the stained oocysts were examined by high pressure mercury arc fluorescent microscopy.

Sporocysts, released from sporulated oocysts by rolling and crushing the oocysts between microscope slides, showed the best differentiation when stained with Acridine Orange solutions of 0.002 and 0.0002 percent.

\textbf{PHASE CONTRAST AND INTERFERENCE MICROSCOPY}

Both phase contrast and interference microscopy have been used in studying the stages of coccidia in sections and in freshly prepared smears of living tissue. The histories and methods of use for both are summarized by Mellors.\textsuperscript{21}

Phase contrast is easier to use and the equipment is much less expensive than the interference microscope. One can see more details in the living endogenous stages with phase than with bright field microscopy. Intravital and other stains are not needed, in most cases. Sporozoites and merozoites are nearly transparent unless phase microscopy is used. When studying material floated on sugar solution, ordinary bright field is more useful than phase microscopy in finding and counting the living oocysts. The phase microscope is needed for studying the internal structures of the oocyst and living intermediate stages, however.

The interference microscope\textsuperscript{1} is costly and takes longer to set up as the color fringes have to be spread to have a uniform color filling the aperture of the objective. This requires the use of the telescope on nearly every slide preparation as each coverslip may be slightly wedged-shaped or it may be tilted so as to form a prism. These adjustments usually have to be made every time one changes to a different power objective. Once the optics are aligned properly, however, the user has most of the advantages of phase combined with the additional ability to produce almost any contrasting color in various parts of the nearly transparent parasite or host cell. One rotates the goniometer scale to produce brilliant colors in the specimens. The expert can measure with the goniometer and apply
the measurements in formulas to determine such things as dry mass, thickness, solid and water concentrations in living cells.\textsuperscript{2} At our laboratory, there has been difficulty in reproducing measurements after moving the goniometer, even when using a half-shade eyepiece to increase the accuracy of the method. Therefore, use of the interference microscope has been limited to qualitative observations instead of quantitative measurements. Used in this manner, it is superior to the phase method.

\section*{HISTOCHEMICAL METHODS}

Sections having low numbers of intermediate stages of coccidia may be stained with various histochemical stains that are specific for cell inclusions predominantly or exclusively located in the parasites but not in the host cells. Giovannola\textsuperscript{11} used polysaccharide stains for observing glycogen in Eimeriae. Edgar \textit{et al.}\textsuperscript{10} found that iodine used alone or in conjunction with a counterstain was useful in checking tissues for isolated oocysts in poultry tissues. Even the youngest uninucleate schizonts, rounded up merozoites or sporozoites in poultry tissues can be detected as colored rings when stained by the Feulgen stain for desoxyribonucleic acid (DNA).\textsuperscript{23} The above methods are also useful in working with the various endogenous stages of coccidia of cattle and sheep.

One of the best of the histochemical stains is that of Himes and Moriger.\textsuperscript{15} They devised a triple stain for demonstrating DNA as a blue or green color, polysaccharides as red, and protein as yellow, all side by side in one tissue section. Trials with this procedure on intermediate stages of \textit{Eimeria alabamensis}, \textit{E. zurnii}, \textit{E. bovis}, and \textit{E. auburnensis} of cattle and of \textit{E. ahsata} of sheep yielded strikingly beautiful preparations.\textsuperscript{4,6,7} The method involves an acid hydrolysis, a Feulgen reaction based on an azure A-Schiff reaction, a bleach, a periodic acid bath followed by a basic fuchsin-Schiff reagent and a naphthol yellow stain for protein.

While the method does not reveal all of the precise structural detail in the cell as well as iron hematoxylin, it makes up for this by making most of the endogenous stages of coccidia stand out in excellent contrast in tissue sections and in smears of intestinal scrapings. When one examines an intestine that may be between 50 and 75 feet long, and the stages are sparse, he can appreciate the benefits of any method that helps to locate the scarce forms by distinguishing them from each other and from the host cells by coloration.

Sections of immature schizonts revealed the nuclei were yellow-green, while at maturity the increased DNA stained a deep blue. Polysaccharides in each merozoite increased in quantity and appeared as minute pink dots. The older the schizont the pinker it became, and eventually this color tone partially masked the blue, giving a distinct color tint to the oldest schizonts.

Microgametocytes were easily distinguished from macrogametocytes by the former appearing multinucleate with the individual nuclei being large and having a greenish color. After cellular division, the nuclei
were smaller and were an intense blue or blue-black. Sometimes the residual masses in microgametocytes were slightly pink. The macrogametocytes are orange or pink while young and turn a bright red as they mature; the greater the amount of oocyst "shell," the redder the contents. The oocyst walls contained a yellow-stained protein. The nuclear material in the macrogametocytes stains poorly.

At the Regional Laboratory, Himes' and Moriber's triple stain is now used routinely, along with iron hematoxylin and hematoxylin and eosin, in studies on life histories of coccidia of cattle and sheep.

SUMMARY

The authors discuss various methods of diagnoses of coccidiosis of cattle and sheep used at the U.S. Regional Animal Disease Laboratory. These include morphological characteristics and location of various endogenous stages. The use of ultraviolet illumination with fluorescent powders or stains is described. They explain the techniques of using phase contrast and interference microscopy and the use of some histochemical methods in studying the parasites.

REFERENCES


Dikmans' checklist\(^1\) of parasites of domestic animals in North America indicates the distribution of over 60 species of helminths that were found in cattle and sheep in the United States before 1945. Since then, additional helminths have been reported in these animals and the distribution of many species included in the checklist has been extended. This paper summarizes some of the more recent reports and information available on the distribution of cestodes, trematodes, and some lesser-known nematodes of cattle and sheep in the contiguous United States.

The economic losses from helminthiasis in domestic animals in the United States were estimated by the U. S. Department of Agriculture\(^2\) in 1954. These estimates were largely based on Federal Meat Inspection records and incidental observations. Because of the paucity of specific data on economic losses involving cattle and sheep, the writer attempted to obtain such data during a three-year period, 1955 to 1958, in Georgia, where helminthiasis is common in cattle and sheep. These data are summarized herein.

**DISTRIBUTION**

**Trematodes**

Price's\(^3\) excellent paper on the trematodes of American ruminants covers the distribution of the species in the United States. His findings are briefly reviewed herein together with additional reports and observations concerning the species found in cattle and sheep.

The common liver fluke occurs abundantly in Florida, Louisiana, Texas, California, Oregon, Washington, Idaho, Nevada, Utah, and Montana, and it has been reported from Arizona, New Mexico, Colorado, Wyoming, Arkansas, Missouri, Michigan, Wisconsin, and Alabama. This parasite has never been known to be established in the Eastern Seaboard States, other than Florida. Federal Meat Inspection records (Table I) show that over 16 percent of the beef livers inspected during 1956 in Florida were condemned for distomiasis, whereas less than one percent were condemned in Alabama, Georgia, South Carolina, and Tennessee. Cattle from Florida, and occasionally from Louisiana and Texas, are shipped to Alabama, Georgia, South Carolina, and Tennessee for slaughter and these animals are probably responsible for the liver condemnations in these four states. In 1957, the liver condemnations in Florida amounted to almost 20 percent, whereas condemnations in abattoirs just across the border in southern

*Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture.*
**TABLE I**

Bovine Livers Condemned for Distomiasis by Federal Meat Inspectors in Abattoirs in the Southeastern States

<table>
<thead>
<tr>
<th>State</th>
<th>Examined</th>
<th>Condemned</th>
<th>Livers</th>
<th>Condemned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef No.</td>
<td>Calves No.</td>
<td>Beef No.</td>
<td>%</td>
</tr>
<tr>
<td>(1956)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alabama</td>
<td>110,146</td>
<td>76,720</td>
<td>456</td>
<td>.41</td>
</tr>
<tr>
<td>Florida</td>
<td>141,035</td>
<td>113,957</td>
<td>22,022</td>
<td>16.25</td>
</tr>
<tr>
<td>Georgia</td>
<td>283,310</td>
<td>148,460</td>
<td>2,187</td>
<td>.77</td>
</tr>
<tr>
<td>S. Carolina</td>
<td>39,771</td>
<td>7,782</td>
<td>186</td>
<td>.47</td>
</tr>
<tr>
<td>Tennessee</td>
<td>66,517</td>
<td>20,162</td>
<td>3</td>
<td>.004</td>
</tr>
<tr>
<td>(1957)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>133,984</td>
<td>148,067</td>
<td>26,743</td>
<td>19.96</td>
</tr>
<tr>
<td>Georgia*</td>
<td>129,683</td>
<td>64,725</td>
<td>597</td>
<td>.46</td>
</tr>
</tbody>
</table>

*Southern Georgia

Georgia were less than one half of one percent. According to meat inspectors in southern Georgia, only Florida cattle harbor liver fluke and this finding coincides with the writer's observations as no flukes were found at necropsy of 61 cattle and 38 sheep from Georgia farms. These results strongly suggest that the common liver fluke is not established in the states named other than Florida.

The fascioloid flukes in American ruminants are considered by most workers as *Fasciola hepatica*; however, there is evidence that a complex of species may exist. In some states, particularly Texas and Florida, flukes have been collected that resemble *F. gigantica*. In addition, flukes also have been found that appear to be a cross between *F. hepatica* and *F. gigantica*, by having the shape of the former and an arrangement of internal organs similar to the latter species. Whether these various forms represent distinct species, or variations of one species is unknown. Considerable work should be done on the progenies of individuals of each kind of fluke to solve this problem.

The large American liver fluke, *Fascioloides magna*, is a parasite of Cervidae and domestic ruminants. It has been reported in Washington, Oregon, California, Montana, Colorado, Kansas, Oklahoma, Texas, Louisiana, Arkansas, Iowa, Minnesota, Wisconsin, Illinois, Michigan, New York, North Carolina, South Carolina, Tennessee, Mississippi, Alabama, and Florida.

The lancet fluke, *Dicrocoelium dendriticum*, which also is a liver parasite, occurs in cattle and sheep, as well as other animals, in New York. This species has been reported in a bovine in Pennsylvania but it is unknown whether the host was a native animal.

Rumen flukes have been collected from domestic ruminants in more than 20 states. Their thick cylindrical body defines specific determination without prepared sections for histologic study. Three species of
Helmints of Ruminants

Paramphistomum have been reported from cattle. Paramphistomum microbothrioides Price and McIntosh, 1944, is the most common species and the U.S. National Museum Helminthological Collection contains specimens from cattle in Virginia, North Carolina, Tennessee, Louisiana, Texas, New Mexico, and Oregon. Paramphistomum cervi is rare and appears to be predominantly distributed in the northern states. Paramphistomum liorchi, a deer parasite, has been reported in cattle in Louisiana and Florida. Cotylophoron noveboracensis Price and McIntosh, 1953, the only species of this genus currently known to occur in this country, is represented by a single record in sheep in New York.

Cestodes

Three species of adult tapeworms occur in cattle and sheep in the United States. The common tapeworms, Moniezia expansa and M. benedeni, are generally distributed in these hosts throughout North America. The fringed tapeworm, Thysanosoma actinioïdes, occurs in the bile duct and small intestine of sheep in the western United States and some North Central States. It has been found as far east as Minnesota, Wisconsin, and Illinois in native sheep.

Five species of immature tapeworms have been reported in cattle and sheep. The thin-necked bladder worm, Cysticercus tenuicollis, the larva of Taenia hydatigena, a tapeworm of canines, occurs on the liver, mesentery, and omentum of domestic and wild ruminants, and is generally distributed throughout North America. Similarly distributed is Cysticercus bovis, "beef measles," which is the larva of Taenia saginata, the beef tapeworm of man. Cysticercus ovis, "sheep measles," the larva of Taenia ovis, has been confused with Taenia krabbei, a parasite of deer and canines, in the western United States. Recent work indicates that T. ovis and T. krabbei are biologically distinct but morphologically indistinguishable. Hydatid, the larva of Echinococcus granulosus, is rare in sheep and cattle but it is occasionally found in these hosts in widely separate parts of the United States. The sheep gid bladder worm, Coenurus cerebralis, which is the larva of Multiceps multiceps, was reported to be widely distributed in Montana in 1910. However, there has been a paucity of additional information on this parasite during the last 40 years.

Nematodes

Recent reports have revealed the presence of some nematodes new to this country and additional reports have extended their distribution along with other lesser-known species of cattle and sheep. Five of the lesser-known nematodes were apparently overlooked because of their similarity to common species and frequent occurrence with them. They are often detectable among numerous nematodes by clearing the worms overnight in lactophenol solution for microscopic examination the following day. A magnification of nine to 54X is usually sufficient to recognize the uncommon ones. Body size, and spicule conformation and length are the usual characters for separation and tentative identification.

Some of the lesser-known nematodes and their known geographic distribution in cattle and sheep in the United States are recorded (Table II).
### TABLE II
The Distribution of Some Lesser-Known Species of Nematodes of Cattle and Sheep in the United States

<table>
<thead>
<tr>
<th>Name</th>
<th>Hosts</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stomach Worms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemonchus similis</em> Travassos, 1914</td>
<td>Cattle</td>
<td>Louisiana,9 Texas and Florida,9 Georgia,10 Alabama,11 North Carolina (U.S.N.M. Helm. Coll.)</td>
</tr>
<tr>
<td><em>Ostertagia bisonis</em> Chapin, 1925</td>
<td>Cattle</td>
<td>Montana,12 Colorado,13 Wyoming14</td>
</tr>
<tr>
<td><em>Ostertagia lyrata</em> Sjoberg, 1926</td>
<td>Cattle</td>
<td>Louisiana,15,16 Georgia,17 Florida,18 Mississippi,19 Oklahoma,20 California,21 North Carolina (U.S.N.M. Helm. Coll.)</td>
</tr>
<tr>
<td><em>Pseudostertagia bullosa</em> (Ransom and Hall, 1912) Orloff, 1933</td>
<td>Sheep</td>
<td>Montana and Colorado,22 South Dakota,23 New Mexico,24 Wyoming,25 Georgia26</td>
</tr>
<tr>
<td><em>Teladorsagia davtiani</em> Andreeva and Satubaldin, 1954</td>
<td>Sheep</td>
<td>California, Connecticut, Georgia, Indiana, Maryland, Missouri, Oregon, Pennsylvania, Vermont, Washington, West Virginia27</td>
</tr>
<tr>
<td><strong>Intestinal Worms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cooperia spatulata</em> Baylis, 1938</td>
<td>Cattle</td>
<td>Georgia,28 Florida,18 Mississippi19</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>Georgia,26 Mississippi19</td>
</tr>
<tr>
<td><em>Cooperia surnabada</em> Antipin, 1931</td>
<td>Cattle</td>
<td>Wyoming,14 Arizona, Florida, Georgia, Oregon, Vermont,29 Oklahoma,20 Iowa30</td>
</tr>
<tr>
<td><em>Cooperia mcmasteri</em> Gordon, 1932</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nematodirus lanceolatus</em> Ault, 1944</td>
<td>Sheep</td>
<td>New Mexico11</td>
</tr>
<tr>
<td><em>Trichostrongylus longispicularis</em> Gordon, 1933</td>
<td>Cattle</td>
<td>Louisiana,32 Florida,33 Georgia,17 Mississippi,19 Oklahoma,20 California34</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>Georgia26</td>
</tr>
</tbody>
</table>

Several of the species are also parasites of wild ruminants. The table and a brief history of five species, with some brief comments on how they may be distinguished from the five common species they closely resemble, are given herein for the convenience of those identifying nematodes at diagnostic laboratories.

*Ostertagia lyrata* Sjoberg, 1926, was reported from cattle in Louisiana in 193015 and 1931,16 and specimens of this species from cattle in North Carolina were deposited in the United States National Museum Helminthological Collection in 1944. In 1958, *O. lyrata* was reported in Georgia17
and later findings revealed it to be common there. This parasite is now known to occur in cattle in Florida, Mississippi, Oklahoma, and California. It is easily confused with *O. ostertagi* with which it is usually found. *Ostertagia lyrata* may be distinguished from *O. ostertagi* by its stout spicules.

*Cooperia spatulata* Baylis, 1938, was first reported in this country in cattle in Georgia in 1958 and subsequently it was found in cattle in Florida, sheep in Georgia, and sheep and cattle in Mississippi. The spicules of *C. spatulata* are similar in conformation to those of the common species *C. punctata*. However, they differ in that those of *C. punctata* are smaller, with a deep concavity in the center which is only represented by a few grooves in the center of the spicules of *C. spatulata*.

*Cooperia surnabada* Antipin, 1931, and *C. mcmasteri* Gordon, 1932 have been synonymized and they are probably conspecific. However, workers have been cautious to accept the synonymy because *C. surnabada* is a Russian species and its type or authenticated specimens are not available for study. These species under one or the other name have been reported in cattle in Wyoming in 1957, Arizona, Florida, Georgia, Oregon, and Vermont in 1958, and more recently in Oklahoma and Iowa. *Cooperia surnabada* and *C. mcmasteri* have been confused with the common species, *C. oncophora*. They may be readily distinguished from *C. oncophora* by spicule conformation in that *C. surnabada* and *C. mcmasteri* have straight spicules, with internal spurs, whose distal ends are capped by triangular membranes, whereas *C. oncophora* has slightly curved spicules, without internal spurs, whose distal ends are capped with oval membranes. There are also marked differences in the dorsal rays between the common and lesser-known two species.

*Trichostrongylus longispicularis* Gordon, 1933, was reported in cattle in Louisiana in 1934, in Florida in 1935, and in Georgia in 1958. Subsequently, a single male specimen was found in sheep in Georgia, and in cattle in Mississippi, Oklahoma, and California. Spicules of *T. longispicularis* are similar to those of the common species *T. colubriformis* and the two species have been confused. The spicules of *T. longispicularis* are very dark, almost black, without distinct barbs near their distal ends, whereas the spicules of *T. colubriformis* are brown or yellow with distinct barbs near their distal ends.

*Teladorsagia davtiani* Andreeva and Satubaldin, 1954, was first reported in this country in 1962, but old nematode collections containing this species from sheep in eleven states, including Alaska, testify to its presence here for over 40 years. The specimens were mistakenly identified as *Ostertagia trifurcata* because the spicules of the two species are almost identical. The presence of an accessory bursal membrane on *Ostertagia trifurcata* and its absence on *Teladorsagia davtiani* distinguish the two species.

**ECONOMIC LOSSES**

Losses from helminthiasis in cattle and sheep largely depend on the prevalence of nematodes, and this in turn primarily depends on prevailing
climatic conditions. Cattle and sheep maintained under different climatic conditions have different nematode populations (Table III). In a small survey of abattoir cattle in the semiarid states of Arizona and New Mexico, only six genera and nine species of nematodes were encountered with the maximum number recovered from any one animal a little over 2,000. In a similar but larger survey of cattle in the humid states of Florida and Georgia, many more genera and species were recovered and the average number of worms encountered was considerably greater than the maximum number found in the cattle from the semiarid states. The humid climate of the Southeast is very favorable for nematode egg development and larval survival on pasture; therefore, southeastern cattle usually have a relatively high worm population. During the spring, summer, and fall, pastures are heavily contaminated with infective larvae and cattle are frequently affected by clinical helminthiasis. The "clinical" cattle in Table III represent the results of a survey involving 32 cattle with clinical helminthiasis in Georgia. These cattle harbored 12 genera and 21 species. The average worm population recovered was approximately 112,000, and the maximum number was approximately a half million.

**TABLE III**

<table>
<thead>
<tr>
<th>Cattle Genera</th>
<th>Species Average Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semiarid States</strong></td>
<td></td>
</tr>
<tr>
<td>New Mexico (abattoir) 6 3 4 . . . 1,255</td>
<td></td>
</tr>
<tr>
<td>Arizona (abattoir) 5 6 9 . . . 2,312</td>
<td></td>
</tr>
<tr>
<td><strong>Humid States</strong></td>
<td></td>
</tr>
<tr>
<td>Florida (abattoir) 20 9 16 13,892 88,227</td>
<td></td>
</tr>
<tr>
<td>Georgia (abattoir) 29 11 18 4,331 22,509</td>
<td></td>
</tr>
<tr>
<td>Georgia (clinical) 32 12 21 111,978 447,212</td>
<td></td>
</tr>
</tbody>
</table>

In an effort to obtain information on the development of the observed helminthiasis and on the economic losses sustained by the owners of the clinically affected cattle, 24 farms where 29 of the cattle originated were visited (Table IV). Morbidity accounted for a little over one-third of the entire cost of helminth parasitism in the animals, death losses accounted for almost two-thirds, and the cost of treatment was between seven percent and eight percent of the total. Although the estimates of economic losses sustained by the cattlemen may appear excessive, they are actually very conservative. Death and morbidity losses caused by parasites
HELMINTHS OF RUMINANTS

TABLE IV
Economic Losses on 24 Farms Caused by Clinical Helminthiasis in Cattle, Including Cost of Anthelmintic Medication

<table>
<thead>
<tr>
<th>Cause</th>
<th>No. Farms</th>
<th>On Farms (No.)</th>
<th>Affected or treated (No.)</th>
<th>Dollar Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Morbidity</td>
<td>19*</td>
<td>6,113</td>
<td>552</td>
<td>9</td>
</tr>
<tr>
<td>Morbidity</td>
<td>24</td>
<td>7,899</td>
<td>270</td>
<td>3.4</td>
</tr>
<tr>
<td>Anthelmintic medications</td>
<td>22**</td>
<td>7,758</td>
<td>4,386***</td>
<td>.</td>
</tr>
<tr>
<td>Morbidity, mortality, and anthelmintic medications</td>
<td>24</td>
<td>7,899</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

*None on five farms on which surviving cattle were thrifty, sold, or dispersed before the information could be obtained.
**None of two farms.
***Received 8,880 treatments.

continued in the herds after the data were obtained and the estimates do not include special feeding and labor costs required for the affected animals.

A survey similar to that on cattle was also completed on sheep. The estimated economic losses from clinical helminthiasis during the survey have not been previously published. During a 32-month study, 21 sheep with clinical helminthiasis were necropsied and the 15 farms where they originated were visited for information pertinent to the survey. Morbidity losses were not estimated because most of the sheep were in full fleece and their physical condition could not be easily determined. The results of the survey (Table V) show that over one-fourth of the sheep on the farms visited died from parasitism. Death losses accounted for approximately four-fifths of the entire loss, and the cost of anthelmintic medications

TABLE V
Economic Losses on 15 Farms in Southern Georgia Caused by Mortality from Clinical Helminthiasis in Sheep and Cost of Anthelmintic Medications

<table>
<thead>
<tr>
<th>Cause</th>
<th>No. Farms</th>
<th>No. on Farms</th>
<th>Affected or treated No.</th>
<th>Dollar Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>Range</td>
</tr>
<tr>
<td>Mortality</td>
<td>15</td>
<td>4,614</td>
<td>1,341</td>
<td>29</td>
</tr>
<tr>
<td>Anthelmintic medications</td>
<td>15</td>
<td>4,614</td>
<td>3,388</td>
<td>73</td>
</tr>
<tr>
<td>Mortality and anthelmintic medications</td>
<td>15</td>
<td>4,614</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
accounted for approximately one-fifth. These estimates are considered conservative for the same reasons indicated for the cattle survey.

The severe losses of sheep from parasitism motivated a survey of the sheep flocks in the Coastal Plain Region of Georgia, roughly the southern half, to determine the extent of losses from helminthiasis sustained by the sheep owners in the region. This was accomplished by obtaining a list of farms with sheep in Georgia from the Georgia Extension Service and randomly selecting 23 (16 percent) farms to be surveyed from the 144 farms with sheep in the region. The 23 farms were widely scattered over the region and their distribution corresponded to that of the sheep population in the region. Between July 1956 and February 1957, each of the 23 farms selected was visited and data on losses from mortality and cost of anthelmintic medications were estimated for the preceding year from information received from the 23 farm owners. Helminthiasis was diagnosed by presence of high fecal worm egg counts, anemia, diarrhea, and other symptoms observed by the owners and veterinarians treating the affected sheep, and at necropsy.

The results of this survey (Table VI) show that (a) approximately 40 percent of the sheep population on the 23 farms visited were on seven farms where almost nine percent died accounting for an average loss of $289, (b) anthelmintic medications costing an average of $167 were given on approximately two-thirds of the farms having 87 percent of the sheep, and (c) the average loss from mortality and cost of anthelmintic medications on all 23 farms was a little over one dollar a sheep per farm. Because these data do not include morbidity and loss of wool and weight they must be considered very conservative estimates of the actual total losses sustained by the farm owners.

The estimates given herein are subject to considerable variation; however, they definitely indicate that helminth parasites of cattle and sheep are an important factor in the economy of cattle and sheep production in the Southeast.

### TABLE VI

| Economic Losses Caused by Mortality from Clinical Helminthiasis in Sheep, Including Cost of Anthelmintic Medications, on 23 Farms Randomly Selected from 144 in Southern Georgia |
|---|---|---|---|---|
| **Cause** | **No. Farms** | **On Farms** | **Affected or Treated** | **Dollar Losses** |
| | | **No.** | **Average** | **No.** | **%** | **Range** | **Average** |
| Mortality | 7* | 1,879 | 268 | 163 | 8.7 | 90-636 | 289 |
| Anthelmintic medications | 17** | 4,116 | 242 | 4,116 | 100 | 7-695 | 167 |
| Mortality and anthelmintic medications | 23 | 4,719 | 205 | . . . | . . . | 14-963 | 211 |

*None on 16 farms.

**None on six farms.
HELMINTHS OF RUMINANTS

REFERENCES

DIFFERENTIAL DIAGNOSIS OF INSECTICIDE POISONING IN LIVESTOCK

R. D. Radeleff*

Kerrville, Texas

Illnesses in livestock characterized by central or peripheral nervous disturbances may be caused by a number of microorganisms, a number of toxic substances, and by such conditions as neoplasias, circulatory stenoses and parasitic invasions. Insecticides are so generally employed for control of insect pests of livestock (occasionally as anthelmintics), and of insects feeding on forage plants, that they are frequently the first to be suspected as the cause of such illnesses.

When the symptoms demonstrated by one or more animals strongly suggest the possibility of insecticide poisoning, there are several points that should be considered before the diagnosis is confirmed. The following suggestions are just that, nothing more. We are not yet able to set down a firm protocol for establishing and confirming a diagnosis of insecticide poisoning.

Suggested Procedure

1. Carefully evaluate the signs of poisoning, the number of animals affected, and the relationship that exists between time of exposure and the appearance of symptoms.

Most of our synthetic organic insecticides act rather rapidly, usually within 12 hours, a few within 24 hours and a rare few within a few days, following exposure. When poisoning is suspected as the result of a single exposure to insecticides a clear line of demarcation between affected and non-affected animals should be apparent within 24 hours. The appearance of new cases on succeeding days should be viewed as an indication that infectious agents should be investigated.

A characteristic pattern of symptoms, compatible with the published descriptions of the symptoms of insecticide poisoning should be in evidence. The presence of an outstanding symptom, such as paralysis of the muscles used in mastication, with resultant dropping of the lower jaw, out-crying with voice change, profuse diarrhea (except in horses), and severe biting, as of an irritated spot, indicate the possibility of infectious diseases or other poisons. Equally, the failure of expected symptoms to appear even though the animal dies, serves as a warning to proceed with care.

2. Determine the true events leading to the situation. When poisoning by insecticides appears undeniable, yet no obvious source of such compounds is discovered, the history of movements of the animals from other premises, where they may have had access to poisons, should be determined.

*Toxicological Investigations, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture.
Frequently, insecticide formulations are used in error instead of anthelmintics and, on occasion, a more toxic material is used than the one intended. Such confusion is most commonly encountered when the owner instructs an illiterate or careless employee to use a specific substance, but fails to verify the action taken.

3. The insecticides leave very few gross lesions, particularly in acute cases; therefore, the presence of significant pathological changes should serve to indicate the probability of some condition other than poisoning.

Unfortunately, a number of diseases afflicting the nervous system also do not leave significant lesions.

4. Establish the identity of the compound employed or to which the animal may have been exposed (see "key"), and establish the maximum exposure that could have occurred.

Before poisoning by any compound can be confirmed we must at least show that there was an opportunity for that compound to reach the animal—and that it was available in sufficient quantity to induce poisoning. This most essential step is so often ignored that unnecessary litigation is the frequent result.

If an insecticidal compound cannot be demonstrated on the premises after exhaustive search, then it is wise to begin to consider other causes. Similarly, if a compound was applied to the animals in dosages well within the safe limits, and particularly when the safety factor is very large, using good technique, it is well to weigh the probability of other causes, including the possibility of potentiation of effects by prior or simultaneous administration of phenothiazine, succinylcholine and similar compounds.

On occasion an owner may be on premises new to him and can give little help concerning suspicious spilled material or the contents of waste dumps or unlabeled containers. In these cases samples of the suspected material should be submitted to a laboratory equipped to analyze pesticides by gas chromatography.

5. Make cautious use of the results of analyses of animal tissues.

There are pitfalls in the interpretation of analyses. Substantial residues of several compounds may be present, depending upon the exposure of the animals, without producing poisoning. These may be present while the actual toxicant is not. With other compounds tissue residues are not developed. Ingesta and hair analyses would reveal recent exposure, but not necessarily to quantities sufficient to kill. The analyses certainly aid in determining the nature of the exposure, but cannot prove intoxication.

Analysis for these compounds in animal tissues is a tedious process unless gross amounts of the particular compound are present. Select reliable laboratories having experience in this field.

The "key" that is presented is offered as a quick reference to help identify specific compounds. It is based primarily on observations of poisoned cattle. I would welcome suggestions for additions or deletions that might be made to improve it.
A POSSIBLE "KEY" FOR SPEEDING IDENTIFICATION OF THE LIKELY COMPOUND IN POISONING BY INSECTIDES

I. Hypersensitive, apprehensive or more alert.

A. Muscular tremors fine and come and go with stimuli at first, later are steady, coarse, and involve eyelids.......................... DDT FAMILY
  Members cannot be distinguished without chemical assistance. Includes DDT, TDE, Methoxychlor, Perthane, Dilan.

B. Muscular spasms, twitches, jerks or convulsions, no steady tremor, may respond to sudden stimuli, excessive salivation mostly froth, may be depressed.................. TOXAPHENE FAMILY
  Onset abrupt, recovery sudden:
  Odor reminiscent of rosin or pine. 
  Musty or somewhat metallic odor.
  BHC or lindane
  Recovery slow:
  Aromatic odor, pleasant, "Tingles".
  Odor absent or not well-defined.

C. Salivation excessive, fluid or stringy, dyspnea, muscular stiffness or weakness, with or without tremors or trembling .......... ORGANOPHOSPHORUS/CARBAMATE FAMILY
  Response to atropine therapy very good.
  Cholinesterase activity markedly depressed a few hours after exposure, salivation, dyspnea, stiff walking main symptoms
  Parathion Group
  Cholinesterase activity returning rapidly to normal. Trichlorfon (Dipterex, Neguvon), DDVP (Vapona), Diazinon, TEPP
  Carbamate Group
  Onset abrupt, course violent, recovery swift. . . Pyrolan
  Onset slower, course extended. . . Sevin
  Cholinesterase activity not depressed, or only slightly, symptoms exaggeration of above, often with tumbling or appearance of rage
  Response to atropine therapy not good, cholinesterase activity continues to decline over several days:
  Central nervous system symptoms not violent. . . Compound 4072
  Central nervous system symptoms violent, alternate with depression. . . Ruelene
  Central nervous system symptoms violent, appear only in terminal state. . . Ruelene

II. Great muscular weakness, inapparent until handled, dragging feet, tail limp. May develop salivation, dyspnea . . . romnel
Organization, personnel, and philosophy are important to the proper function of a veterinary medical laboratory. However, other specific factors also must be considered.

An adequate technical program must be conceived and developed after the laboratory has been logically organized and appropriately staffed. Predetermined objectives of the laboratory, the geographic location, and the extent and nature of the research program will influence the technical activities, the personnel and equipment needs. One other essential consideration is the acquisition and reporting of accurate data from a given disease situation as rapidly and economically as possible. The quality of work and service rendered by the laboratory are dependent to a significant degree on the scientific disciplines maintained and the instrumentation used to execute them. It is to this subject the present discussion is addressed.

The present sophisticated state and rapid advancements being made in the scientific arts make an aggressive methods and instrumentation program mandatory. Laboratory objectives, local needs, and available personnel vary widely between institutions. Therefore, as a premise for this discussion, it is assumed a typical veterinary medical laboratory with adequate physical plant facilities will be organized and staffed as outlined before this group on previous occasions.1,2

Specific laboratory instrumentation must logically be considered in association with scientific disciplines, techniques, and personnel. Instruments should be considered and justified for addition to the laboratory armamentarium. Measuring the need for the equipment by the following criteria will prove useful:

1. Will the instrument perform techniques and produce useful data not now obtainable?
2. Will it permit an improvement of techniques and increase the amount, value and/or reliability of pertinent data?
3. Will it perform the desired volume of work with less manpower and therefore be more economic than present equipment?

The Veterinary Pathologist

Since the pathologist gives overall direction to the technical laboratory activities, he must have general orientation and capabilities in most procedures used.

*Pathology Department, Pitman-Moore Division, The Dow Chemical Company.
Specifically, a modern bright-field microscope designed for the pathologist is a basic instrument. This includes a variety of easily applied light filters and polarizing attachments for special applications.

A microscope specifically designed and equipped for phase-contrast microscopy is desirable for maximum usefulness in this work. If this is not possible, a dual purpose microscope may be selected as a second choice. Phase-contrast techniques are extremely useful in studying unstained preparations. Tissue culture cells, small parasites and many other similar preparations lend themselves to this technique.

Fluorescence has now been developed to a high degree of sophistication and every laboratory would do well to have a microscope equipped and adjusted for this work. Vital staining of tissue culture cells, blood cells, and certain animal tissue cell preparations is a useful procedure and these may be profitably studied by this technique. The identification of antigen particles by fluorescent antibody methods are now in common usage in many laboratories. Facilities for making good quality photomicrographs of each of these instruments should be provided so that valuable temporary specimens can be made a permanent record.

**Microbiology Section**

The inter-related disciplines of pathogenic bacteriology, mycology, virology and serology will be discussed as an integrated operation. At least two constant-temperature incubators are needed for pathogenic bacteriology and mycology. One of these may be permanently adjusted to 37.5°C, while the other may be used to provide varying temperature as needed.

A good commercial hatching incubator with dependable temperature and humidity controls is required for egg culture. The size can be adapted to individual laboratory needs.

For routine tissue cell culture, an incubator is needed that reliably maintains required temperatures within narrow ranges and should be provided with a recording thermometer.

A constant-temperature incubator in which carbon dioxide tension and humidity can be maintained and controlled is required for certain specialized tissue culture techniques.

Routine tissue culture procedures should include virus isolation, characterization, and identification, as well as serum-antibody titration. A variety of media, primary cells, virus strains, and known antisera is necessary for these functions. Continuous stable cell culture lines are often useful in this work. Essentially the same procedures will also be conducted in embryonating eggs.

An inverted-type microscope especially designed for studying tissue cell culture layers in bottles and tubes is highly desirable. This instrument provides greater objective to condenser distance and good depth of field in focus.

The microbiology section will have need for both bright field and darkfield microscopes. A dissecting microscope will be helpful in the examination of chick embryos, membranes, small bacterial colonies, and
other materials not suited to routine microscopy. One microscope each for phase-contrast and fluorescence studies are usually adequate for the entire average laboratory, but an active microbiology section will find many uses for these instruments.

A mechanical low-temperature freezer is preferred over the solidified-carbon-dioxide chest for preserving live virus material because of economy of operation and reduced hazard to living viral agents. Additional equipment of specialized nature is essential to the microbiologist and can be supplied as needed. A large variety of media must be maintained for differential pathogenic bacteriology and moderate variations in media are required for mycology and tissue culture.

**Histopathology Section**

The two most frequently-used methods for preparing histologic tissue sections are paraffin infiltration and freezing.

The methods and instruments used most widely for preparation of paraffin sections are well known. Precision microtomes, automatic tissue processors, and mechanical microtome knife sharpeners are routinely used in most histopathology laboratories. Automated instruments may be used to stain tissue sections by routine methods such as hematoxylin and eosin, but most special stains are best done by hand.

The microtome-cryostat is of fairly recent development and has now been perfected to a high state of refinement. Sections of sufficient quality to permit the most exacting examination are possible. Tissues may be frozen and sectioned fresh to permit critical differential staining including procedures for certain enzymes and other specific material. This instrument is a definite asset in any histopathology laboratory.

Differential and histochemical techniques are essential for maximum service from histopathology. A wide range of useful and practical staining techniques are now in general use. Stock materials should be maintained and many special stains can be prepared and stored in the refrigerator for instant use.

**Clinical Pathology Section**

Many functions in clinical pathology require fairly routine equipment such as the bright field microscope, clinical centrifuge, water baths, slide staining facilities and other simple apparatus.

One of the most useful instruments for blood chemistry, organ function tests, and many other techniques is the spectrophotometer. These instruments are versatile, and accurate. Standardized procedures for conducting the most commonly used tests are available. A flame photometer might be provided either as an attachment to the spectrophotometer or as a separate instrument if tests for trace metals are to be run in significant numbers. In the event large numbers of blood chemical tests are to be done, the spectrophotometer is available as an automated instrument which conducts and prints out the results of as many as 60 or more tests per hour.
If large numbers of erythrocyte or leukocyte counts are to be done, such as might be required in certain research work, an automated electronic counter is a fast and accurate instrument that will materially increase the efficiency of technicians' time.

Prothrombin tests, microhematocrit, sedimentation rates and similar procedures are essential and the equipment is not elaborate or expensive.

Clinical Toxicology Section

Due to the excessive cost of equipping and operating a credible clinical toxicology function, a veterinary medical laboratory rarely will maintain such a unit. A laboratory is usually well advised to make arrangements with their state toxicologist or some other well equipped laboratory with personnel trained and experienced in clinical toxicology to conduct the necessary tests. Other laboratory results may guide and support the work in clinical toxicology regardless of where it is done. In any case, each institution must supply the equipment and trained personnel necessary to meet the needs and objectives of the individual laboratory.

RECORDS AND DATA

It is necessary to devise a record system that is simple and as "error proof" as possible. The system must be strictly executed and short cuts cannot be indulged by anyone. If laxities are permitted, a breakdown will inevitably occur and the system will soon become essentially useless or even worse than none at all.

Well designed work sheets for each laboratory section are helpful in consistently obtaining a maximum of useful information from each case or experiment. Work sheets for each section may be color coded for ready recognition and speed in handling.

An efficient system for recording, storing and retrieving data must be developed if maximum value is to be obtained from the field cases. Seasonal incidence, geographic distribution and other epizootiological data is readily available when an efficient system is established.

If the services of an automated computer are available locally or even at some distance, this equipment can profitably be utilized for storing and retrieving data. Equipment is now in general use that can provide this service at reasonable cost. Specialists at the computer center can assist in devising a program that will most nearly meet individual laboratory needs.

If the services of automated computer equipment are not available, a more simple system using cards single or double punched along their margin may prove adequate. These systems are simple, but time-consuming for office personnel and therefore more expensive unless the case load is small.
DISCUSSION

After the objectives of the veterinary medical laboratory have been established, the type and quantity of data required to meet these goals must be defined. Instruments best suited to the needs can then be measured by the criteria mentioned above. Where heavy work loads are the rule, highly refined, automated instruments are usually more accurate and economical to use. Quality of data must never be compromised. Annual case loads will be considered in staffing and equipping a laboratory. Highly capable and dependable personnel are required, but fewer total people are needed with good instrumentation. Good instrumentation usually speeds availability of results and therefore makes the study more useful to the practicing veterinarian and the animal owner.

Prudent business procedures are ethical and practical necessities in both public and private institutions.

SUMMARY

1. The laboratory case load and the research program will influence the scientific disciplines, specialization of the staff, and the instruments required for optimum function.
2. Creditable dispatch in obtaining and reporting accurate data must be a constant objective.
3. With a large field case load and/or an active research program good instrumentation usually becomes a scientific and economic necessity.
4. Good business principles and practices are compelling obligations in both private and public institutions.

REFERENCES

A BIO-ASSAY FOR MYCOPLASMA GALLISEPTICUM

C. H. Bigland and A. J. DaMassa
University of California, Davis, California

Needed for a confirmatory diagnosis of *Mycoplasma gallisepticum* infection in turkeys and chickens with sinusitis, airsacculitis, coryza, and tracheitis is a combination of typical history of slow spread, positive serology, culture, and bird inoculation. The usual tests are as follows:

1. *M. gallisepticum* serum or whole-blood agglutination test\(^1,3,6\) or hemagglutination inhibition test\(^6,9,10\)
2. Culture on solid media,\(^6\) broth,\(^4,17\) or broth overlay.\(^6\)
3. Injection of suspect material into the sinuses of turkeys\(^15\) or the respiratory tracts of birds immune to IB and NCD and free of *M. gallisepticum* infection.\(^8\)

Serology is rapid but gives no indication of an active disease process, and culture and bird inoculation are lengthy and often inconsistent with serological results. Moreover, *M. gallisepticum* cannot be identified with certainty from morphology or biochemical reactions. Proof usually requires preparing an antigen or inoculation into birds. The following test procedure determines the identity of the organisms; is relatively rapid, providing evidence of viable organisms in a few days, is specific for *M. gallisepticum*, and is extremely sensitive.

**HISTORY**

Injection of cultures of *M. gallisepticum* into the air sacs regularly causes airsacculitis,\(^11,14,18\) and exudates from the sinuses of turkeys infected with *M. gallisepticum* will also produce a severe airsacculitis in chickens and turkey poults.\(^19\) McMartin and Adler\(^16\) found the minimal infective dose of *M. gallisepticum* injected into the air sacs of chickens to be \(10^{1.8}\) organisms. Ten days after injection, serum agglutination titers ranged from 1:10 to 1:160. The following experiments were designed to evaluate air sac inoculation of chicks or poults with sinusitis exudates, turbinate and air sac tissue suspensions, or *M. gallisepticum* cultures as a rapid, reliable test for viable *M. gallisepticum*. The progress of infection was followed by serological testing, necropsy, and reisolation of the organism.

**MATERIALS AND METHODS**

*Experimental birds*

Birds inoculated were: (1) young Leghorn chickens, three to 16 weeks old, from a flock free of *M. gallisepticum*, and (2) Broadbreasted Bronze turkeys, 12 weeks old, originating from a flock tested clean on a voluntary *M. gallisepticum* agglutination testing program.
Procedure

The air sacs of chickens and turkeys were injected with 0.5 ml. amounts of: (1) dilutions of *M. gallisepticum* culture, (2) saline suspension of ground air sac or turbinate tissue from turkeys inoculated intranasally with *M. gallisepticum*, (3) diluted or undiluted sinus exudate from naturally infected turkeys. Stained *M. gallisepticum* antigen was used for serological tests. Material for culture was inoculated on PPLO agar plates (Difco) to which had been added 10 percent horse serum, PPLO broth overlay, or carbohydrate broth media.

PROCEDURE AND RESULTS

Experiment 1

To confirm McMartin and Adler's observations and relate serological responses elicited by injection of sinus exudate with the response to a known number of *M. gallisepticum* organisms, 0.5 ml. of different dilutions of *M. gallisepticum* culture, originally titrated at $10^9$ organisms per ml., was injected into the abdominal air sacs of chickens. By the fifth day post-inoculation the sera from all chickens injected with the highest concentration of organisms ($5 \times 10^7$) displayed detectable titers, and a serologic response was evident in all reacting birds by the 10th day post-inoculation (Table I). All chickens with positive titers exhibited air sac lesions at necropsy (12 days post-inoculation). In one instance (bird 15), airsacculitis and *Mycoplasma* recovery were observed in the absence of an agglutinin titer, possibly indicating that tissue damage precedes establishment of an immune response. The lowest number of organisms eliciting a response was $5 \times 10^1$ or $10^{1.69}$, which compares favorably with McMartin and Adler's AID$_{50}$ of $10^{1.8}$. Recovery of a *Mycoplasma* that was presumably *M. gallisepticum* from air sac lesions was consistent with carbohydrate media but inconsistent with direct plating.

Experiment 2

Sinus exudate was obtained from 12 mature turkeys with clinical evidence of sinusitis, caused by *M. gallisepticum* (S$_6$ strain) intranasal instillation, and with serum agglutination titers of 1:16-1:128. Undiluted sinus exudate from each donor (0.5 ml.) was injected into the abdominal air sacs of two chickens. Exudate from each of five of the donors was also injected into the abdominal air sacs of two young turkeys, to compare the two species in serological response and air sac reaction. Serological responses were detected by the 8th post-inoculation day in all turkeys, and by the 11th day in chickens (Table II). In some instances in both species, titers were observable by the seventh day. All chickens and turkeys exhibiting a serological response also had severe air sac lesions. A *Mycoplasma* was recovered from the affected air sacs on agar plate or broth overlay from 19 of 21 chickens and nine of 10 turkeys cultured.
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<th>Sugars Plate</th>
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</tr>
</tbody>
</table>

@ 0.5 ml. into abdominal air sacs
* Leghorn chickens nine weeks old from M. gallisepticum - free flock
b Twelve days post inoculation
S Suspicious
U Undiluted
X Not tested
- Negative
TABLE II
Examination of Chickens and Turkeys Following Injection into Air Sacs of 0.5 ml Undiluted Sinus Exudate from Turkeys with Gross Sinusitis from *M. gallisepticum* (5-6) Intranasal Instillation

<table>
<thead>
<tr>
<th>Donor Turkeys</th>
<th>Experimental Results in Chickens &amp; Turkeys&lt;sup&gt;(d)&lt;/sup&gt;</th>
<th>Day 1st Sera Necropsy isolation&lt;sup&gt;(b)&lt;/sup&gt; Titer at&lt;sup&gt;(a)&lt;/sup&gt; (12 days)</th>
<th>Mycoplasma Chicken</th>
<th>Turkey</th>
<th>Plate</th>
<th>Broth&lt;sup&gt;(e)&lt;/sup&gt;</th>
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<td></td>
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<td>Chicken</td>
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<td>A 64 Bilat. ++++</td>
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<td>8</td>
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<td>16</td>
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<tr>
<td>B 64 Left ++++</td>
<td>2 died</td>
<td>8</td>
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<td>32</td>
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</table>

(a) Severe air sac lesions at point of inoculation in all injected birds - No lesions in controls.
(b) Air sacs cultured: plate - direct on PPLO (Difco) agar + 10 percent horse serum broth - overlay broth on agar
(c) Mycoplasma - not *M. gallisepticum*
(d) Leghorn chickens seven weeks old from *M. gallisepticum* free flock, BBB turkeys 12 weeks old
(e) Isolation through broth not attempted if plate isolations positive.
(x) Not cultured
A BIO-ASSAY FOR MYCOPLASMA GALLISEPTICUM

TABLE III
Serological and Necropsy Examination of Chickens Injected with Air Sacs and Turbinate Tissues from Turkeys Infected with *M. gallisepticum* (S-6 Strain) but Exhibiting no Gross Sinusitis

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<thead>
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<th>Donor Turkey(b)</th>
<th>Experimental Chicken(d)</th>
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</table>

(a) Tracheal rales at 11 days.
(b) Mycoplasma cultures negative on plate and overlay broth.
(c) Direct plating - noninhibitory PPLO serum agar.
(d) Leghorn chickens - 35 days old - from *M. gallisepticum*-free flock.

**T** - Trachea  **AS** - Air sacs  **U** - Undiluted

Experiment 3

A challenge for the bio-assay technique was provided by turkeys showing little or no evidence of sinusitis or respiratory distress 52 days after intranasal instillation of *M. gallisepticum* (S₆ strain). Turbinate and
air sac tissues were taken from six donor turkeys exhibiting a slight turbinate exudate, slight sinusitis, or air sac lesions. These were ground with sterile saline, and 0.5 ml. of each tissue suspension was injected into the air sacs of two chickens. Serological reactions were evident in chickens injected with tissues from five of the six donor turkeys (Table III). The first serological responses were noted on the 9th day post-inoculation, and nine of 11 reacting birds were positive by the 13th day post-inoculation, although two chickens remained negative until the 17th and 19th days. Air sac lesions were present in all reacting birds. On culture, a *Mycoplasma* was recovered from six of the nine affected air sacs. A serological response was evident in eight of 12 chickens injected with turbinate tissues, but in only three of 12 chickens injected with air sac tissues from the same donors, indicating that the organism, in concentrations detectable by this method, was most often confined to the turbinate. Tracheal rales were noted in two inoculated chickens at 11 days post-inoculation. These were probably due to *M. gallisepticum* invasion, since Newcastle disease HI tests of all chickens and infectious bronchitis SN tests of nine were negative at the beginning and end of the test.

**Experiment 4**

To evaluate the effect of injecting sinus exudate taken from turkeys suffering from infectious sinusitis in the field, exudate from three adult affected birds from two separate flocks was injected into two young chickens from each sinus exudate or dilution used (Table IV). These responded serologically in seven to 12 days, and severe air sac lesions were noted at autopsy. In no instance could *Mycoplasma* be isolated from donor turkeys or inoculated chickens, and donor turkey "A" had no demonstrable agglutinin titer. Other instances of sinusitis in turkeys with negative agglutinin titer and isolation have been noted at this and other laboratories.

**DISCUSSION**

The injection of *M. gallisepticum* cultures or exudate from birds with either artificial or natural infection of *M. gallisepticum* causes a rapid specific serological response to *M. gallisepticum* antigen, accompanied in this test by air sac lesions or tissue damage. McMartin and Adler, using dilutions down to $10^{-8}$ organisms, consistently observed a serological response with *M. gallisepticum*-induced air sac lesions. In Experiment 1, $10^{-1.69}$ *M. gallisepticum* elicited a positive serological response. Since so few organisms can be detected by this method, it should be useful in assaysing turkey sinus exudate, chicken nasal exudate, chicken or turkey turbinate tissues, or joint fluid or other tissues suspected of harboring *M. gallisepticum*, and even in detecting *M. gallisepticum* carriers that are not exhibiting clinical symptoms. The positive results with a dilution of turkey sinus exudate of 1:100 in Experiment 4 indicate that even tiny amounts of exudate could contain detectable numbers of *M. gallisepticum*. Considering the occasional difference in response of chickens injected with
TABLE IV
Response of Chickens Following Air Sac Inoculation with Sinus Exudate from Field Cases of Turkey Sinusitis

<table>
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<tr>
<th>Donor Turkeys</th>
<th>Experimental Results in Chickens&lt;sup&gt;(e)&lt;/sup&gt;</th>
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</thead>
<tbody>
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<tr>
<td>A(d)</td>
<td>Neg.</td>
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<tr>
<td>A</td>
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<tr>
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</tr>
<tr>
<td>B</td>
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</tr>
<tr>
<td>B</td>
<td>1:64</td>
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</tr>
<tr>
<td>C</td>
<td>1:4</td>
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<tr>
<td>Controls. Inoc. with 0.5 ml.</td>
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<tr>
<td>Sterile broth</td>
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</tr>
</tbody>
</table>

<sup>@</sup> Severe air sac lesions at point of inoculation in all injected birds. No lesions in control birds.

<sup>(b)</sup> Tom - bilateral sinusitis - clear exudate - many coccobacillary bodies.

<sup>(c)</sup> Hen - bilateral sinusitis - cloudy exudate - few coccobacillary bodies.

<sup>(b+c)</sup> From flock 3600 nine months old - 70 percent coughing, 30 percent sinusitis. No *M. gallisepticum* titer at three, 12 and 26 weeks.

<sup>(d)</sup> Few cases sinusitis in flock of origin. No. *M. gallisepticum* titer in random 10 percent flock.

<sup>(e)</sup> Leghorns from *M. gallisepticum* free flock: ages one to two (eight weeks), three to six (16 weeks), seven to 10 (three weeks).

<sup>+</sup> Positive but not titrated.

The culture of *M. gallisepticum* from clinical cases of chronic respiratory disease of chickens or infectious sinusitis in turkeys has been traditionally inconsistent<sup>2</sup> and unreliable. *M. gallisepticum* of the S<sub>6</sub> strain used in Experiments 1, 2 and 3, has been adapted to growth on artificial media since its isolation from turkey brain, in 1954<sup>20</sup>. Such adaptation may be the reason that the S<sub>6</sub> strain could be recovered readily from the affected air sacs of injected chickens and turkeys although no *M. gallisepticum* could be isolated from the air sacs of test chickens inoculated with sinus exudate from field cases (Table IV).

The serological response of chickens and turkeys to *M. gallisepticum* following injection into the air sacs is consistent, elicited rapidly, and highly sensitive, rendering the test satisfactory for laboratory and field use. Either chickens or turkeys can be used. If chickens free of *M.
*Mycoplasma gallisepticum* are not available, turkeys negative to the *M. gallisepticum* agglutination test could be used. In the field, such turkeys, even from the same flock, would be satisfactory, the injected birds revealing detectable titers in at least ten days, with concurrent airsacculitis at the site of injection. The latter procedure would eliminate the necessity to maintain test birds on another premise.

In applying the test, serological reactions can be confirmed by necropsy of test chickens and turkeys and observation of air sac lesions at the site of inoculation.

The bio-assay technique could be used for the identification of *M. gallisepticum* cultures and possibly other *Mycoplasma* species if confusion exists in colony identification. It may also be used for testing the efficacy of drug treatments, as suggested by Adler *et al.*

**SUMMARY**

Inoculation into the air sacs of young chickens and turkeys of *Mycoplasma gallisepticum* cultures, *M. gallisepticum*-infected turbinate and air sac tissue suspensions, and sinus exudate from natural and artificial *M. gallisepticum* sinusitis in turkeys, produced serum titers detectable by *M. gallisepticum* plate agglutination antigen in three to 19 days post-inoculation, with gross air sac lesions occurring at the point of inoculation. This technique could be used for detecting *M. gallisepticum* in any suspect tissue or material such as sinus exudate in turkeys, nasal or joint exudates, turbinates, and lung or tracheal tissues of chickens, and differentiation of *M. gallisepticum* from other *Mycoplasma* in cultures. Under field conditions, a suspicion of *M. gallisepticum* sinusitis could be confirmed utilizing turkeys on the same ranch.

**ACKNOWLEDGEMENTS**

The suggestions and manuscript review of Drs. H. E. Adler and R. Yamamoto, the assistance of Dr. W. W. Sadler and R. E. Corstvet and the provision of field materials by Drs. R. Mitchell and W. M. Dungan are sincerely appreciated.

**REFERENCES**

A BIO-ASSAY FOR MYCOPLASMA GALLISEPTICUM


Scrapie is an insidious, chronic, degenerative disease of sheep producing lesions in the central nervous system. It is manifested clinically by signs of pruritus, locomotor disturbances, and hypersensitivity or marked dullness. It has been recognized in Europe for over 200 years. The first case in North America was reported from Canada in 1938. The first reported case occurred in the United States in 1947. Since then scrapie has spread to many parts of the country. In 1952, it was introduced into New Zealand. It has recently been reported in India.

The incubation period of experimental scrapie is commonly four to 22 months. The most prominent histopathologic change is vacuolation of neurons in the brain stem and spinal cord to a degree not approached in any other disease of animals or man.

The cause of scrapie is still debated. One group believes natural scrapie is due to a single autosomal recessive gene. However, they concede that it can be artificially transmitted to some sheep, which may not have the correct genetic composition for the natural development of scrapie, as well as to goats and mice. They have adapted the term "provirus" for what they consider to be a genetically determined but artificially transmissible scrapie agent.

Other workers, including those at Moredun Institute and Compton Research Station, believe scrapie is an infectious disease due to a self-replicating agent. The findings of Eklund, Hadlow and Kennedy suggest that the scrapie agent is not a member of a unique group of pathogens but is a medium-sized virus whose resistance to heat is analogous to that of serum hepatitis virus. Neither prolonged boiling nor treatment with ribonuclease or deoxyribonuclease inactivates the inoculum. Experimental oral transmission of scrapie to sheep and goats is reported. There is good evidence of natural spread of the disease in sheep and goats under controlled experimental conditions.

Antibodies against scrapie have not yet been demonstrated. Changes in serum proteins have not been detected. Muscular lesions associated with scrapie have been compared with dermatomyositis and muscular dystrophy of humans. However, subsequent communications report examination of numerous scrapie animals in which muscular lesions were not observed consistently. One of these workers concluded that scrapie cannot be considered a primary disease of the muscle, and that muscle changes play no apparent part in producing clinical symptoms of that disease.

From the Pathology Section, Diagnostic Services, National Animal Disease Laboratory (NADL), Ames, Iowa.
Vacuolation of neurons is the most spectacular lesion of scrapie. Stamp states, "The vacuolated neurons, however, are of special interest as due to their great numbers and multiplicity of forms they are very characteristic if not indeed pathogenonomic for scrapie." Nevertheless, vacuolated neurons do occur to some extent in normal sheep. Numerous studies have been designed to differentiate scrapie affected sheep from normal sheep by differences in both morphologic features and number of vacuolated neurons. Brownlee found vacuolated neurons in the brains of each of 24 typical cases of scrapie, and none in the central nervous system of 64 normal sheep. Holman and Pattison examined 75 scrapie sheep and 98 normal sheep. They found vacuolated neurons in all of the scrapie-affected animals and in five normal animals. They concluded that, "clinical symptoms suggestive of scrapie followed by microscopic discovery of vacuolated nerve cells in the medulla oblongata probably constitute sufficient evidence on which to base a diagnosis of the disease." Pattison later reconfirmed this diagnostic criteria and extended the significance of vacuolated neurons to scrapie affected goats. The clinical signs and lesions in the brains of mice artificially inoculated with scrapie have some similarities to the clinical signs and lesions in scrapie-affected sheep and goats.

A comprehensive study by Palmer discussed numbers and morphology of vacuolated neurons in both normal and scrapie-affected sheep as compared to those found in other infectious diseases, parasitic conditions, trauma, and other diseases of sheep.

Zlotnik and Rennie have investigated morphology, numbers and locations of vacuolated neurons in normal and scrapie-affected sheep. They examined 180 serial sections from the medullas of each of 57 apparently normal sheep of various breeds, mostly Chevoits. Vacuolated neurons were found in 73.7 percent of the medullas. As many as seven vacuolated neurons per microscopic field were found in a few sheep. The majority of vacuolated neurons in non-scrapie sheep were in the dorsal motor nucleus of the vagus nerve.

The authors extended their studies to include 60 additional normal sheep, 29 of which were from the so-called scrapie-free area of the United States. Breeds of sheep from the United States included 10 crossbreds, 10 Colombias, eight Rambouillets, and one Hampshire. Zlotnik and Rennie concluded that vacuolated neurons can be found in the medulla of apparently normal sheep, including breeds in which natural scrapie has not been reported. However, the number of vacuoles occurring in the medulla is low, and only in exceptional cases exceeds one per section.

Zlotnik published the results of histopathologic studies and vacuole counts comparing the brain stems of 70 sheep affected with natural scrapie and 70 apparently normal sheep. He classified the vacuolated neurons according to the following six types: (1) Single vacuoles in otherwise healthy neurons, (2) multiple vacuoles, usually small, in otherwise normal neurons, (3) single vacuoles in degenerating neurons, (4) many small vacuoles in cell bodies or axons of degenerating cells, (5) multilocular vacuoles in cells undergoing chromatolysis, and (6) large single or multiple vacuoles
in necrotic cells. Only the Type 1 vacuolated neurons were found in normal sheep. The presence of the other five were limited to scrapie sheep.

Zlotnik summarized his observations by stating that: "The number of vacuoles present in 54 serial sections from three levels of the medulla of non-scrapie animals did not exceed 100 and were usually much less, while in scrapie animals the corresponding figure was at least 400, and more often, well over 1,000."

**Eradication Measures**

The scrapie eradication program in the United States requires slaughter of, and allows indemnity for, all sheep and goats in affected flocks, source flocks, exposed animals moved from these flocks, and their immediate progeny. Criteria for the diagnosis of scrapie must be derived from definitive changes which have been described by competent, trained observers.

The purpose of this paper is to relate our experiences and outline the laboratory procedures employed in the diagnosis of scrapie at the Diagnostic Services Section, National Animal Disease Laboratory (NADL). This is the reference laboratory for the national scrapie eradication program.

The study reported here is based on the examination of 125 specimens from scrapie-suspicious animals received from January 1, 1959, to July 1, 1963. A positive diagnosis was rendered in 58 cases. Many of the specimens were examined histologically by laboratories other than NADL. In most instances, the sheep were necropsied at locations away from NADL and the brain specimen submitted by mail from veterinarians in the Animal Disease Eradication Division, State Diagnostic Laboratories, Colleges of Veterinary Medicine, and in practice. However, on several occasions, one of the authors (H.A.M.) has performed the necropsy and collected specimens for histologic study.

**Clinical Signs**

The case histories submitted with the 125 brain specimens from scrapie-suspicious sheep were reviewed. Many of these were scanty. Such statements as, "This sheep showed classical symptoms of scrapie, examine it histologically" appeared to often. However, some of the case histories were thorough and complete. The following clinical manifestations stand out as meaningful.

Pruritus and locomotor disturbance appear to be the most diagnostic symptoms. Scrapie seldom occurs in animals under 18 months old. All of the scrapie cases which we examined were in sheep more than two years of age. Another characteristic of scrapie is its prolonged course. In our cases, the duration of the overt clinical disease always exceeded 30 days.

When the case histories are studied individually, a high degree of correlation can be seen between pruritus and disturbances in locomotion of 30 days or more duration in sheep two years old or older and the microscopic changes in the brain which are associated with scrapie. Examination
of scrapie positive case histories revealed that thirty-four of the sheep were over two years old and manifested pruritus and locomotor disturbance for longer than 30 days, whereas only one negative case filled all these criteria. Many of the other 24 positive cases probably fit in this category, but the case histories failed to mention at least one of these factors. It is realized that one or more of the clinical manifestations of scrapie may not be observed in certain cases of scrapie. Nevertheless, the occurrence of pruritus and locomotor disturbances that last 30 days or longer in sheep two years old or older in strong evidence of scrapie.

The first sign of scrapie is loss of wool in many cases. Rubbing and scratching vigorously against any convenient object and biting and gnawing upon the wool and skin may be observed. In addition, hyperexcitability, incoordination, slight tremors of the head and neck, rapid muscular tremors of the thighs and flanks, loss of weight, dry lusterless wool, and lagging behind the rest of the flock may be noted. These manifestations are frequently noticed by the owner or caretaker and, hopefully, he will call a veterinarian.

The veterinarian should determine the presence or absence of cutaneous acariasis by examining scrapings of the affected skin under the microscope. Diseases affecting the nervous system that may be confused with scrapie include listeriosis, pregnancy disease, pseudorabies, and rabies. Disturbances in locomotion and coordination can usually be detected and evaluated by making the animal walk backward, more to the side, step or jump over obstacles 12 to 18 inches high, and make sharp turns. These exercises are more meaningful if several other sheep are made to perform along with the suspect. Sheep affected with scrapie may assume an unusual stance or an abnormal position of the head, such as holding the head in a slightly distended or rotated position, especially when the "scratch reflex" is elicited.

This "scratch reflex" is another classic feature of scrapie. It is elicited by briskly rubbing or scratching the animal's back and is manifested by nibbling motions of the lips, rapid viper-like movements of the tongue, and wiggling of the tail stump. However, the "scratch reflex" may be absent in scrapie or may be present in other diseases.

In all cases, the duration of each clinical sign, as well as the age, sex, breed, source, and registration number should be recorded.

Once the suspicion of scrapie is aroused, state or federal veterinary regulatory officials should be contacted. Diplomacy by the regulatory veterinarian is needed. The owner should be encouraged to let the sheep live until it recovers or death appears certain. If the sheep recovers, it can be concluded that the animal did not have scrapie. Nevertheless, additional inspections should be made from time to time over a period of six months due to possible remission of scrapie signs. Scrapie sheep will probably live at least 30 days after manifesting symptoms. Death usually follows in two to six months. Whenever possible the sheep should be moved to a state diagnostic laboratory or school of veterinary medicine where it can be observed several times each day and necropsied in the terminal stages of the disease or immediately following death.
The importance of close observation of the suspicious animal and advance preparations for necropsy cannot be overemphasized. Of the 125 cases examined at our laboratory, 17 of the tissue specimens had undergone severe post-mortem degeneration. Ten had been frozen either before fixation or while in transit. Autolytic changes were prominent in seven.

Before the sheep dies, a source of dry ice or deep freeze facilities, 15 percent neutral buffered formalin, and adequate instruments to remove the brain and open the spinal canal should be assembled.

Post-Mortem Examination and Laboratory Procedures

Lesions diagnostic of scrapie are only apparent in the central nervous system on microscopic examination. Nevertheless, a thorough examination of the entire carcass should be made and the findings recorded.

The instruments and gloves used in removing the brain should be heat sterilized. The frozen tissue specimen will often be used for tissue culture and laboratory animal inoculation.

The brain is removed and processed as follows:

1. Remove the head at the atlanto-occipital joint.
2. Remove the skin from the top of the skull and posterior part of nose, including the skin around the eyes. (See Figure 1).
3. Using a post-mortem saw, or similar device, make cut "A" to extend from a point one-half inch posterior to the middle of the orbit to a similar point posterior to the opposite orbit (See Figures 2

Figure 1. Note the skin reflected from the midline and cut "A" traversing the forehead.
Figures 2 and 3. Diagramatic sketches of the two cuts necessary to open the cranium.

4. Cut "B" is made on each side of the cranium from the middle of the foramen magnum to a point one inch medial to the orbital rim, cut "A" and cut "B" bisecting at this point. (See Figures 3 and 4). This cut should be angled about 45 degrees inward.

Figure 4. Note cut "B" joining the foramen magnum, and the chisel used to pry off the top of the skull.

Figure 5. Both cuts "A" and "B" are discernible, the "skull cap" is reflected anteriorly. The dura mater has been removed from the surface of the brain.
5. Insert a heavy knife, chisel, or similar object in cut "B", and pry the top of the skull forward. (See Figures 4 and 5). Precautions should be taken at this step to prevent the attached meninges from disrupting the brain substance, especially the tentorium cerebelli. A good pair of surgical scissors are recommended for cutting these membranes.

6. Cut the olfactory tract and raise the brain slightly upward so the optic nerves can be cut. (See Figure 6). As the brain is raised, the pituitary stalk comes into view. The stalk is cut leaving the pituitary gland in its bony fossa.

7. The brain is gently raised upward and backward. By cutting the cranial nerve roots, the brain is freed from the cranial cavity.

NOTE: The removal of a brain intact requires a delicate and exacting technique. The technique as stated here is not the only procedure and will probably be varied somewhat by each individual. The important point is to get the brain out and shipped with as little contamination, distortion, and laceration as possible.

8. The vertebral arches along the entire length of the spinal column are then cut away with bone forceps, and the spinal cord is carefully examined for abscesses or other lesions.

9. The cerebral cortex should be carefully separated from the brain stem with a pair of scissors and thumb forceps and frozen immediately. (See Figure 7). The brain stem should be placed in a container with approximately two quarts of 10 to 15 percent neutral formalin and the cerebral cortex frozen.

Figure 6. The optic nerves are being cut (1), the olfactory tracts have been bisected previously. The brain is being reflected dorsally and posteriorly out of the cranium.

Figure 7. The brain stem and cerebellum on the right (1), are separated from the cerebral cortex (2). The brain stem and cerebellum should be fixed in formalin and the cerebral cortex frozen.
buffered formalin. (In no instance should the brain stem be frozen, since it is destined for histologic examination.)

10. After the brain tissue has been fixed in formalin 24 hours, it can be immersed in 10 percent formalin in a four to eight oz. specimen container and forwarded to a laboratory equipped for histologic examination.* The cerebral cortex should be packed in dry ice and forwarded to the same laboratory. Formalin fixed tissue should not be included in the package with the dry ice unless a shipping carton constructed specifically for this purpose is used.

The following selection of blocks for histologic study, based largely on the work of Zlotnik and recommended by Dr. J. T. Stamp,** is followed where applicable at NADL. (See Figure 8).

Figure 8. Blocks cut from the brain stem and cerebellum which are to be dehydrated, cleared and embedded in paraffin. They are from the following:
1. Thalamus including the mamillary body.
2. Mesencephalon including the anterior colliculus and pineal body.
3. Mesencephalon including the inferior colliculus.
4. Pons and cerebellum.
5. Medulla including the trapezoid body.
6. Middle medulla.
7. Posterior medulla.

*If specimens are sent to the Diagnostic Services section of NADL, they should be addressed as follows: Dr. E. E. Wedman, Chief Veterinarian, Diagnostic Services, National Animal Disease Laboratory, P.O. Box 70, Ames, Iowa.
**Personal communication.
1) One block through the thalamus to include the mamillary bodies.
2) Two blocks through the mesencephalon to include the red nucleus.
3) One block through the pons and cerebellum.
4) Three blocks from the medulla (one block to include the trapezoid body, one through the middle, one through the posterior medulla).
5) One block of the medulla-cord junction.

11. The following sections are cut at seven microns, mounted, stained, and examined microscopically.

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>six consecutive sections</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>54 serial sections from each block of the mesencephalon (every third one is mounted)</td>
</tr>
<tr>
<td>Pons, medulla, and</td>
<td>18 consecutive serial sections from each block</td>
</tr>
<tr>
<td>medulla cord junction</td>
<td></td>
</tr>
</tbody>
</table>

(A total of 132 sections are mounted for microscopic examination.)

**Diagnosis**

Microscopic changes in the brain stem consist of vacuolated neurons, progressive degeneration of neurons, astrocytosis, and vacuolation of the neuroparenchyma which imparts a spongy appearance to the neuroparenchyma. (See Figures 9-14). The most readily recognized change in routine

Figure 9. Single vacuole in an otherwise healthy cell classified by Zlotnik as type 1. This is reportedly the only type vacuolated neuron encountered in normal sheep.
Figure 10. Scrapie sheep brain: Note pyknotic neurons (1), normal neuron (2), and multiloculated vacuolated neurons (3).

Figure 11. Scrapie sheep, brain stem, note vacuolated neuron containing intravacuolar material (1); pyknotic neurons (2); multilocular vacuoles in vacuolated neurons (3); and normal neuron (4).
Figure 12. Scrapie sheep brain stem. Note multivacuolated pyknotic neuron (1) adjacent to a normal neuron (2). Other degenerated and normal neurons are observed among prominent microglia and oligodendrocytes.

Figure 13. Scrapie sheep, cerebellum. Note the ground substance vacuolation in the molecular layer (1) and to a lesser extent in the outer granular layer. Pyknotic Purkinje cells (2) are also discernible.
preparations is neuronal vacuolation, which Stamp suggests is very characteristic if not indeed pathognomonic for scrapie. These large holes in the cytoplasm appears to result from an outwardly directed force. The Nissl substance, nuclei, and other intracellular components are often pushed to one side of the cell body. Frequently the vacuole is multilocular and resembles a clump of bubbles. Eosinophilic round bodies may be observed inside the vacuole. We frequently find more than 50 vacuolated neurons per section of medulla in positive scrapie cases. Vacuolated neurons are usually concentrated near the midline and distributed in a bilaterally symmetrical pattern, i.e., if a specific nuclear group of neurons is severely affected, the corresponding group on the opposite side of the brain will be similarly affected. In addition, neuronal degeneration characterized by pyknosis, chromatolysis (tigrolysis), and other degenerative changes occur in vacuolated and non-vacuolated neurons (See Figures 10-12).

The neuroparenchyma may also be vacuolated, especially in the cerebellum (Figures 13 and 14). The vacuolation may be diffuse (Figure 13), but more often occurs among groups of vacuolated neurons. Although some of the neuroparenchymal vacuoles appear to result from vacuolated neurons in which only the neuronal cell wall remains, this is not always the case. There are often more vacuoles in a given area than can be accounted for by the multiplicity of neurons expected in a similar location in normal sheep. Zlotnik believes edema accounts for many of the extracellular vacuoles.
One of the most significant tissue alterations reported to date is astrocytosis. This cellular change allowed a correlation between scrapie and the human disease, Kuru. However, in our opinion, a diagnosis of scrapie can be made without the special staining and impregnation necessary to adequately demonstrate the astrocytosis.

In most cases, it is not necessary to examine the entire 132 sections of brain stem. One or two sections from each block may suffice. Either the history does not indicate scrapie, i.e., reported course of a week or less, or other lesions sufficient to account for death of the sheep are disclosed. For example, clinical internal parasitism may result in a chronic course and arouse suspicion of scrapie. The post-mortem examination discloses the parasitism, but the possibility of scrapie may still exist in the mind of the owner. In such cases, the same procedure is followed except only two or four sections are mounted from each paraffin block. Edema is usually the only change observed microscopically in such brain tissue.

A diagnosis of scrapie should be made when the clinical manifestations and duration of the disease indicate scrapie, and when vacuolation and degeneration of neurons in the brain stem is in accord with the findings of Zlotnik and other research workers. If the number of vacuolated neurons is large, i.e., 30 or more per section throughout the brain stem and the total count in 54 sections of medulla will exceed 400, confirmation of the clinical diagnosis of scrapie is possible without examining all the sections. Most of the positive cases presented to NADL are in this category; 50 or more vacuolated neurons per section is the usual finding and over 100 per section is not uncommon. If the number of vacuolated neurons is small or portions of the brain stem show no lesions, the complete examination should be made.

The work of Zlotnik serves as an excellent guideline in these cases. He reported the numbers of vacuolated neurons did not exceed 100 in 54 sections of medulla from normal sheep, and that his examinations never disclosed less than 400 vacuolated neurons in 54 sections of medulla from scrapie affected sheep.

REFERENCES

THE DIAGNOSIS OF SCRAPIE 563


LABORATORY CONSULTATION AND EVALUATION OF ROUTINE LABORATORY STUDIES ON SWINE DISEASES

F. E. Mitchell, D.V.M., M.S., and V. B. Robinson, D.V.M., Ph.D.*
Indianapolis, Indiana

This subject encompasses a major objective for most veterinary medical laboratories. We would, therefore, not claim that our approach to laboratory consultation is different or superior to the operation of most laboratories, but we feel it is effective in providing essential information necessary for the definitive diagnosis of major swine diseases. Definitive in this sense means to us a diagnosis which is certain or probable beyond reasonable doubt. A minimum of time to obtain this information and little direction to the laboratory workup by the attending pathologist are desirable features to this approach. Cases are submitted only by a veterinarian as he is the individual best qualified to apply laboratory results to the respective clinical problem. Modest charges are made for these laboratory services. These factors have a selective effect on the type of cases submitted and discourage those which do not warrant the time and expense of laboratory work.

I. Laboratory Activities

A. Case records. Clinical information important to the case is recorded on a master record form and laboratory work sheets which are color coded to facilitate quick identification and distribution to the respective laboratory units. Each sheet provides appropriate spaces to record results of the case workup in each unit.

B. Necropsy. Euthanasia, dissection, collecting blood for hemograms and tissues for the microbiologic and histopathologic examinations are all responsibilities of an animal technician. An assistant helps at necropsy by setting up specimen containers, labeling, and recording observations. This individual can also trim tissues directly from the carcass to a size suitable for receptacles for overnight processing thereby making the histologic examination possible the next day. Duplicate formalin preserved tissues as denoted in Figure 1 are also routinely saved in the event other histologic sections may be needed. After dissection by the technician the pathologist is summoned to dictate notes on gross observations and to give special instructions when desired.

C. Microbiology, serology, clinical pathology, and histology. Swine cases usually require no more than conventional laboratory techniques from these disciplines. The laboratory building provides individual laboratory units for these activities and each unit is staffed with medical technologists and experienced microbiologists whose work can be relied upon.

From the Pathology Department, Pitman-Moore Division, The Dow Chemical Company.
with confidence. Adequately trained and competent laboratory personnel in these areas minimize a need for direct supervision, thereby freeing the pathologist for work of a more specialized nature.

D. Animal inoculation, toxicology and tissue cell culture. Animal inoculation and clinical toxicologic tests are usually employed on special occasions when more definitive evidence is needed and earlier clinical laboratory information provides only a presumptive diagnosis.

A well qualified clinical toxicology laboratory located nearby in another institution is utilized for the few cases which call for this type of laboratory investigation. Through this laboratory we are able to provide reliable toxicologic service with expert opinion without the disadvantages of maintaining the staff and apparatus in our own department necessary for this seldom used but important activity. It serves further to help exclude our laboratory personnel from being involved in litigation which these cases so often require.

Tissue cell culture has been used widely as an exploratory tool in field cases during the past two years, but it has contributed essentially no information of diagnostic value on the major swine diseases.

E. File systems. A simple system using the McBee Keysort Card is used to record significant details from clinical and laboratory information. The punched cards store data and it is relatively simple to retrieve information even among large numbers of case records.

The laboratory work sheets and a copy of the laboratory reports are bound and stored in permanent files for reference in the event details of a particular case are needed at a later date.

Figure 1. Pig carcass depicting tissues collected routinely during dissection for the histopathologic examinations.
II. Evaluation of Laboratory Studies

A. Diseases studied. Table I gives a summary of the principal diseases diagnosed among 2900 swine cases in a four year period. The 30 percent that are not listed include undiagnosed cases, vibriosis, parakeratosis, erythrozoan infection and other diseases which occurred at a much lower incidence.

**TABLE I**
Principal Diseases among 2900 Cases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cases ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hog Cholera</td>
<td>668 (23.0%)</td>
</tr>
<tr>
<td>Mixed Bacterial Septicemia</td>
<td>330 (11.4%)</td>
</tr>
<tr>
<td>VPP and Septic Bronchopneumonia</td>
<td>232 (8.0%)</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>212 (7.3%)</td>
</tr>
<tr>
<td>Streptococcal Disease</td>
<td>210 (7.2%)</td>
</tr>
<tr>
<td>Septicemia</td>
<td>117 (4.0%)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>26 (0.9%)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>39 (1.3%)</td>
</tr>
<tr>
<td>Other</td>
<td>28 (0.9%)</td>
</tr>
<tr>
<td>Enterotoxemia</td>
<td>170 (5.8%)</td>
</tr>
<tr>
<td>Poisoning</td>
<td>88 (3.0%)</td>
</tr>
<tr>
<td>Chemical</td>
<td>53 (1.8%)</td>
</tr>
<tr>
<td>Moldy Feed</td>
<td>14 (0.4%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>21 (0.7%)</td>
</tr>
<tr>
<td>TGE</td>
<td>65 (2.2%)</td>
</tr>
<tr>
<td>HC and Salmonellosis</td>
<td>35 (1.2%)</td>
</tr>
<tr>
<td>CASES</td>
<td>2,010 (69.1%)</td>
</tr>
</tbody>
</table>

B. Necropsy results. Generally, gross lesions of infectious diseases in pigs are non-specific and of no more value than to suggest the possible presence or absence of a septicemic disease.

C. Clinical pathology. The major contribution to the differentiation and diagnosis of bacterial and viral disease is the leukocyte count. In certain cases, however, atypical and unexpected white blood cell counts may occur as shown by the results in Figure 2. The best safeguard against being misled by the atypical count is the selection of several rather than a single individual from the herd for these blood cell counts.

Hemoglobin, hematocrit and erythrocyte values are helpful in cases of iron deficiency and other anemias.

D. Serologic tests. Worthwhile information on infectious diseases is limited mostly to brucellosis and leptospirosis. Analysis for vitamin A may give significant information on rare occasions.

E. Cultures for bacterial pathogens. The value to be derived from these procedures is apparent from the list of bacterial diseases among the entities in Table I. In final analysis, however, interpretation of culture results must be correlated with histopathologic findings in order to reach a reliable opinion.

F. Histopathologic studies. In our experience this procedure provides the most effective means by which rapid and dependable diagnostic evidence
of swine diseases can be obtained. The most distinguishing histopathologic characteristics of the more important swine diseases which we have studied are discussed with accompanying photomicrographs.

1. Hog cholera. The presence of a disseminated aseptic vasculitis widespread in the central nervous system is the principal criterion for suspecting this disease. Perivascular accumulation of lymphoid cells and focal aggregates of neuroglia (Figure 3) are the principal components and these are simple indications of neurologic viral activity. The other diseases in this country that produce reasonably similar reactions are rabies, viral polioencephalomyelitis and Aujeszky's disease; therefore, diagnosis is usually reduced to a minor problem after an aseptic encephalitis is recognized.
Figure 3. Aseptic encephalomyelitis characteristic for hog cholera virus activity, a) brain, b) spinal cord. X 75
Figure 4. Exudative lesions with mixed bacterial septicemia, a) lymph node abscess, b) umbilical abscess. X 75
The association of a characteristic disseminated aseptic meningoencephalomyelitis with hog cholera has been confirmed by pig inoculation tests on numerous occasions in our laboratory.

2. **Mixed systemic bacterial infections.** This designation is used as a diagnosis for cases in which exudative and/or necrotizing lesions (Figure 4) are seen in more than one organ as the principal pathologic manifestations along with the recovery of two or more genera of bacteria in association with the lesions. These include streptococci, staphylococci, Corynebacterium, Pseudomonas, Pasteurella and coliforms. These infections occur with a frequency sufficient to suggest a primary disease syndrome. The incidence of mixed infections was highest in newborn pigs.

3. **Virus pig pneumonia (VPP) and septic bronchopneumonia.** These two entities occurred together so consistently they essentially form a single disease of dual etiology with VPP probably being the initial irritant. The condition often occurred along with a more important disease problem, but in eight percent of cases this was judged as the primary disease syndrome. The complex usually appears histologically as an exudative reaction superimposed on alveolar septal cell proliferation and peribronchiolar lymphoid hyperplasia (Figure 5).

---

**Figure 5.** Lymphoid hyperplasia (1) and bronchiolar exudate (2) with VPP and septic bronchopneumonia. X 75
4. *Salmonellosis.* Histopathologic examination of the liver regularly reveals discrete foci of coagulation necrosis and moderate attraction of inflammatory cells (Figure 6). Other less meaningful microscopic lesions include septic lymphadenitis, bronchopneumonia and exudative enteritis.

![Figure 6. Hepatic focal necrosis and septic inflammation with salmonellosis. X 75](image)

5. *Hog cholera and salmonellosis.* Recognition of these concurrent infections is based on the separate and distinct histopathologic changes mentioned above and poses no great problem.

6. *Streptococcal infection.* This infection seems to involve four distinct anatomical locations to a significant degree. These infections may be present singly, or in combinations of organs. The inflammatory process (Figure 7) may be either acute or chronic.

a. Meningitis . . . Here the reaction is usually exudative and purulent.

b. Arthritis . . . Acute cases are seen as regular septic reaction with fibrin and/or purulent exudate on the joint surfaces; chronic reactions produce fibrosis and greatly enlarged joints.
c. Pleuritis-pericarditis... Acute suppurative reaction predominates, but a chronic mixed proliferative and exudative response may be seen.

d. Pharyngeal lymphadenitis or the "jowl abscess" is another frequent lesion.

All of these occur with considerable frequency and together they have accounted for about 7.2 percent of cases in this review.

7. Enterotoxemia. This entity is identified by deduction as much as by association with specific lesions. For this reason, greater emphasis is placed on the importance of thorough laboratory studies in order to confidently exclude other diseases. The basic histologic lesions (Figure 8) are edema of the meninges and perivascular spaces of the brain with mild exudation of mixed types of inflammatory cells. However, this is not always sufficiently developed to give the pathologist confidence in his interpretation. Similar but less meaningful alteration of vascular permeability is seen microscopically, and occasionally grossly, in other organs. We have gained no definite impression from our experience as to the cause of this entity, but the reaction is compatible with acute allergic response.

8. Poisoning. Clinical proof of exposure to the suspected poison is necessary to establish a diagnosis, though microscopic lesions (Figure 9) may provide suggestive evidence of the nature of the toxicity. Eosinophilic encephalomeningitis of salt poisoning and moldy feed encephalomalacia are two of the more important histopathologic clues which we encounter.

9. TGE. There is difficulty in providing definitive diagnosis of this disease histologically or by any other laboratory means. This is due to several factors which include: a) peracute deaths of highly susceptible newborn pigs occur before lesions have time to develop; b) isolation and identification of the infectious agent is not easily accomplished by present laboratory methods, and, c) gastroenteritis may result from other causes and therefore is nonspecific.

Considerable confidence in a diagnosis of TGE, however, can be had if there are edema and round cell infiltration of intestine mucosa (Figure 10) coupled with a clinical picture of high morbidity and mortality of baby pigs while older animals on the premises are showing diarrhea and vomiting.

DISCUSSION

The purpose of this presentation is to emphasize that a thorough laboratory study usually can provide a rapid and definitive diagnosis in most field cases of disease in swine. Though various methods serve their purpose, it is our feeling that greatest single benefit is derived from histopathologic procedures. Since early symptoms and gross lesions of the major swine diseases are not definitive, a reliable diagnosis is not possible without thorough laboratory studies. This applies equally to field cases and investigational work. Unfortunately the literature on hog cholera investigation, for example, contains many reports that have confused
Figure 7. Exudative a) meningitis, b) synovitis, c) pleuritis with streptococcal infections. X 75

Figure 8. Distended perivascular spaces contain few, mixed types of inflammatory cells, reaction associated with enterotoxemia. X 75
Figure 9. a) Eosinophilic encephalitis characteristic of salt poisoning. X 75
b) Mold toxicosis encephalomalacia. X 75
rather than clarified the issues largely because of inadequate laboratory studies of experimentally diseased animals.

Our laboratory operation permits an evaluation of the presence or absence of hog cholera with confidence in about 24 hours and the professional competence and facilities required to provide this service are not prohibitive. Failures would be expected only when unsatisfactory materials were presented for study.

SUMMARY

The main activities related to a laboratory consultation service on swine cases are outlined. These include a records system and investigative methods, such as necropsy, microbiologic, histopathologic and clinical pathology examinations.

The value of information to be derived from the respective examinations is discussed with greatest emphasis placed on histopathologic findings.
ANTIBODY RESPONSE OF TURKEYS EXPOSED TO
MYCOPLASMA GALLISEPTICUM

R. Yamamoto, Ph.D.:* W. E. Babcock, D.V.M., M.S.:*
and E. M. Dickinson, D.V.M., M.S.

Most work concerned with the detection of antibodies to *Mycoplasma
gallisepticum* in turkeys has been limited to determining the response of
birds at one particular time after infection.\(^1,2,3,4\) Some have dealt with the
number of birds reacting positive or negative over an interval.\(^5,6\) While
Hofstad\(^7\) studied the antibody response of turkeys to *M. gallisepticum*
infection over a 21 month period, the first testing was not initiated until ap-
proximately two months after symptoms were observed and was continued
only at irregular periods thereafter. Nevertheless, much valuable infor-
mation was obtained as to the interpretation of the tube agglutination (TA)
and hemagglutination-inhibition (HI) tests.

Recently, Adler, *et al.* (1962)\(^8\) showed a high correlation between posi-
tive titers and the presence of symptoms or lesions. The HI, TA, and
plate agglutination (PA) procedures were equally satisfactory. These find-
ings were based on necropsy and serology five weeks after intranasal ex-
posure of turkeys to *M. gallisepticum*.

The present study used the plate agglutination test to follow more
closely the antibody response patterns in turkeys exposed to *M. gallisepti-
cum*. Birds were exposed via the intrasinus route, the air sac route, and
through contact with birds so inoculated.

While the antibody response was studied at all stages of infection,
particular attention was given to the early period in order to detect the in-
itial response and its rise to peak titer.

Attempts were made to relate the differences in antibody response
patterns among different exposure groups to clinical signs, lesions, and
cultural results. The contact exposure phase of the study yielded additional
information on this mode of transmission of the agent.

MATERIALS AND METHODS

*Antigen preparation and standardization.* The antigen was prepared es-
tentially by the method of Adler and Yamamoto.\(^9\) Purified *M. gallisepti-
cum* strain S6 was received in a lyophilized form from Dr. H. E. Adler.
The maintenance medium consisted of PPLO broth (Difco) containing yeast
hydrolysate,\(^*\) one percent; horse serum, 10 percent; and penicillin, 1,000

Technical paper No. 1744 from the Oregon Agricultural Experiment Station,
Department of Veterinary Medicine, Corvallis, Oregon.

*Present address: Yamamoto, Department of Avian Medicine, University of
California, Davis, California; Babcock, Chas. Pfizer & Co., Terre Haute, Indiana.
**Nutritional Biochemical Corporation.

578
units/ml. The medium for antigen preparation contained glucose, 0.05 percent, as an additional supplement.

Cultures were incubated for seven days and then harvested by centrifugation at 10,000 x g for 10 minutes. The pellet was washed once and resuspended in saline buffered with Sorensen’s buffer, pH 7.0. This buffered saline contained 0.25 percent phenol as a preservative.

The antigen was standardized on a Bausch & Lomb Spectronic 20 at 650μ. An optical density of 0.125 of a 1:10 dilution of the antigen was considered as the standard density; this was equivalent to 10 X #1 McFarland nephelometer. The standardized antigen was used without the addition of dyes. The sensitivity and specificity of antigens, checked with known positive and negative turkey sera, showed very little difference from harvest to harvest.

Plate agglutination test. It was found by experimentation that the rapid-plate pullorum testing procedure, routinely used in this laboratory, could be adapted for large scale testing for Mycoplasma. Glass plates, four by 13 inches were marked off into 16 squares in a single row. The antigen was deposited within the squares with a dropper calibrated to deliver 0.05 ml. per drop. A volume of 0.01 ml. of serum was expelled from a 0.05 ml. pipette onto the antigen drop and mixed gently with the tip. The pipette was rinsed in saline and wiped with a gauze between samples when a series of tests was performed. The glass plate was placed over a fluorescent light on a dark counter and the agglutination reaction determined.

Doubling dilutions of serum in saline were used for titration; 0.01 ml. of each dilution was mixed with 0.05 ml. of antigen. Titers were recorded as the highest dilution of serum which reacted with the antigen in a specified time period. The reaction was considered positive as long as definite agglutination was observed. For any one group of turkeys at a particular bleeding period, the geometric mean titer (GMT) was calculated.11,12

In the acute stage of the disease, the agglutination reaction of positive sera could be visualized readily within three minutes after the tests had been set. Therefore, during this period the tests were read after a three- to five-minute reaction time. However, with clinical recovery, a longer reaction time was required before agglutination of positive sera occurred. Therefore, all samples tested from the 19th week after the experiment was initiated, were set and read at three, five and 10 minutes. From these results it was found that a five minute holding period prior to reading gave satisfactory differentiation between sera of different reactivity. Therefore titers were recorded on the basis of the five-minute test.

Inoculum. Strain 1056 of M. gallisepticum was used to infect turkeys. This strain, originally recovered from a chicken, consistently produced sinusitis in experimentally inoculated turkeys in earlier studies.

Turkeys. Beltsville White, Broad White, and Broad Breasted Bronze turkeys, six months of age or older unless indicated otherwise, were obtained from the Department of Poultry Husbandry. This closed flock was negative for clinical signs and for M. gallisepticum agglutinins when tested
periodically. Turkeys slaughtered from this flock were free of airsacculitis. All turkeys were pretested for *M. gallisepticum* agglutinins prior to use.

**Experimental design.** Ten adult turkeys, designated sinus principals, were inoculated with 0.5 ml. (0.5 X 10<sup>8.83</sup> ELD<sub>50</sub>) of infected yolk per right sinus and placed in a floor unit, 8 by 10 1/2 feet. Ten additional turkeys of the same age, designated air sac principals, were inoculated via the left abdominal air sac with one ml. of inoculum (10<sup>8.83</sup> ELD<sub>50</sub>) and placed in a similar unit.

Uninoculated turkeys, five per group, were placed in contact with each of the principal groups at different times and held for varying intervals. A particular contact group was killed when transmission, as evidenced by clinical signs and antibody response, occurred; a new group of turkeys then was placed in contact with the principal group. This procedure was repeated until contact transmission had ceased, as indicated by lack of clinical signs, and by absence of antibody response. Two contact groups (I and II) were placed in contact with the air sac principals; seven (I through VII), with the sinus principals.

All birds were bled at weekly intervals until peak titers (GMT) were obtained; thereafter, bleeding was done at irregular intervals. Clinical signs and general conditions were noted. At necropsy, lesions were scored and cultures taken for *Mycoplasma* isolation. During the course of the study, sinus exudate was taken from representative birds with sinusitis to determine the persistence of the organism. *Mycoplasma* recovered from birds with a prolonged clinical course were tested for pathogenicity by sinus inoculation in normal turkeys.

**RESULTS**

**Response of principal groups.** Birds inoculated via the sinus route responded with a higher titer than did birds inoculated by the air sac route (Figure 1). This marked difference in antibody response patterns of the two groups was confirmed in another trial (not shown).

Rales were detected in six of the air sac principals two weeks post-inoculation (p.i.); by four weeks all birds in this group were showing this sign. After the 12th week, clinical signs were no longer evident. Table I gives the necropsy findings of these birds killed 23 weeks p.i.

In the sinus principals, nine of 10 birds had sinusitis one week p.i. Rales were detected two weeks p.i., and subsequently detected in the greatest number of birds (5/10) five weeks p.i. Only one bird exhibited rales beyond 11 weeks which persisted through the 18th week. General condition of the birds appeared improved by nine weeks, and by 13 weeks the sinusitis was regressing in the majority of the turkeys. However, when killed between 34 and 70 weeks p.i., many still had sinusitis (Table II).

**CONTACT TRANSMISSION STUDIES**

**Air sac principals.** Group I of the controls was placed in contact with the principals at the time the latter were inoculated. The antibody response is
Figure 1. Antibody response of turkeys exposed to *Mycoplasma gallisepticum* via the sinus (sinus principals), via air sac (air sac principals), and by contact with the respective principals at intervals indicated.

**TABLE I**

Turkeys Inoculated with *Mycoplasma gallisepticum* Via the Air Sac (Air Sac Principals): Observations at Necropsy, 23 Weeks after Inoculation

<table>
<thead>
<tr>
<th>Bird No.</th>
<th>Air Sac Lesion Score*</th>
<th>Agglutination Titer</th>
<th>Isolation** (Air Sac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>411</td>
<td>1</td>
<td>1:2</td>
<td>-</td>
</tr>
<tr>
<td>412</td>
<td>1</td>
<td>neg.*</td>
<td>-</td>
</tr>
<tr>
<td>413</td>
<td>2</td>
<td>1:2</td>
<td>-</td>
</tr>
<tr>
<td>414</td>
<td>1</td>
<td>1:2</td>
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</tr>
<tr>
<td>415</td>
<td>1</td>
<td>1:4</td>
<td>ND</td>
</tr>
<tr>
<td>416</td>
<td>2</td>
<td>1:8</td>
<td>/</td>
</tr>
<tr>
<td>417</td>
<td>2</td>
<td>1:8</td>
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<tr>
<td>418</td>
<td>2</td>
<td>1:4</td>
<td>/</td>
</tr>
<tr>
<td>419</td>
<td>1</td>
<td>1:1</td>
<td>-</td>
</tr>
<tr>
<td>420</td>
<td>1</td>
<td>1:16</td>
<td>ND</td>
</tr>
</tbody>
</table>

*The turkeys were in good flesh and were not exhibiting clinical signs; Lesion score 1; consolidated lesion; 2; consolidated lesion plus a small amount of exudate. No other lesions were evident.

**ND, not done; air sac tissues were passaged through chicken embryos prior to plating.
TABLE II

Turkeys Inoculated with Mycoplasma Gallisepticum Via the Sinus (Sinus Principals): Observations at Necropsy

<table>
<thead>
<tr>
<th>Bird No.</th>
<th>Time of Slaughter*</th>
<th>Lesions**</th>
<th>Isolation (Air Sac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>410</td>
<td>34</td>
<td>±</td>
<td>1:32</td>
</tr>
<tr>
<td>401</td>
<td>36</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>403</td>
<td>36</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>405</td>
<td>36</td>
<td>±</td>
<td>1:4</td>
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<td>406</td>
<td>36</td>
<td>-</td>
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<td>409</td>
<td>36</td>
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</tr>
<tr>
<td>404***</td>
<td>69</td>
<td>±(-)</td>
<td>1:64(1:32)</td>
</tr>
<tr>
<td>407</td>
<td>69</td>
<td>±</td>
<td>1:32</td>
</tr>
<tr>
<td>408</td>
<td>69</td>
<td>±</td>
<td>1:4</td>
</tr>
<tr>
<td>402</td>
<td>70</td>
<td>-(-)</td>
<td>1:32(1:32)</td>
</tr>
</tbody>
</table>

*Weeks after inoculation; at necropsy, all turkeys were in good flesh, and with the exception of those with chronic sinusitis, showed no clinical signs.

**Si, sinusitis; AS, air sacculitis; S, air sac lesion score, all were consolidated, see Table I for interpretation.

***Turkeys, 404 and 402 were reinoculated with pathogenic Mycoplasma via the sinus 68 weeks after initial injection; (), sinusitis or agglutination titer at the time of reinoculation.

shown in Figure 1. Rales were detected in one of five birds at five weeks post-contact (p.c.) and in four birds at eight weeks. At five weeks p.c. one bird had sinusitis. In all of the contact experiments including those of the sinus principals, this was the only bird that developed sinusitis. Necropsy findings for this group are shown in Table III.

Group II of the controls was placed in contact with the principals 11 weeks after the latter had been inoculated. At this time the principals had a GMT of 1:16 and one bird was showing rales. These controls remained clinically and serologically negative for a 10-week contact period (Figure 1). At necropsy the birds were in good condition and had no gross lesions.

**Sinus principals.** Birds inoculated by the sinus route remained infectious for an extended period after inoculation. Consequently, seven contact controls (Groups I through VII) were studied over a 67-week period. The antibody response patterns of these groups are shown in Figures 1 and 2.

Rales were first detected in the group I contacts at two weeks p.c., and subsequently in the greatest number of birds six weeks p.c. The antibody response is shown in Figure 1. The birds were killed and examined at 11 weeks p.c. (Table III). Rales appeared in the group II contacts at two weeks p.c.; by five weeks the majority were showing signs. Rales were not observed between eight and 10 weeks p.c., at which latter time the turkeys were killed. Necropsy results are presented in Table III. The antibody response is shown in Figure 1.
TABLE III
Necropsy Findings of Turkeys Infected by Contact with the Sinus or Air Sac Principals

<table>
<thead>
<tr>
<th>Contact Group</th>
<th>Contact Interval (Weeks)**</th>
<th>Bird No.</th>
<th>Lesions***</th>
<th>A.S. Lesion Score****</th>
<th>Titer</th>
<th>Isolation***** (Air Sac)</th>
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</thead>
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<tr>
<td>I AS*</td>
<td>0-11</td>
<td>1</td>
<td>+ + + +</td>
<td>3</td>
<td>1:16</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+ + + +</td>
<td>3</td>
<td>1:4</td>
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<tr>
<td></td>
<td></td>
<td>3</td>
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<td>+ + + +</td>
<td>3</td>
<td>1:16</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>+ + + +</td>
<td>3</td>
<td>1:16</td>
<td>-</td>
</tr>
<tr>
<td>I Sinus</td>
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<td>6</td>
<td>+ + -</td>
<td>2</td>
<td>1:256</td>
<td>-</td>
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<td></td>
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<td>7</td>
<td>+ + + +</td>
<td>2</td>
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<td></td>
<td></td>
<td>8</td>
<td>+ + +</td>
<td>2</td>
<td>1:64</td>
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<td></td>
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<td></td>
<td></td>
<td>10</td>
<td>+ + +</td>
<td>2</td>
<td>1:32</td>
<td>-</td>
</tr>
<tr>
<td>II Sinus</td>
<td>11-21</td>
<td>11</td>
<td>- + -</td>
<td>1</td>
<td>-</td>
<td>ND</td>
</tr>
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<td></td>
<td></td>
<td>12</td>
<td>- + +</td>
<td>2</td>
<td>1:8</td>
<td>+</td>
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<td>15</td>
<td>- + -</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>III Sinus</td>
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<td>- - -</td>
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<td>24</td>
<td>- + +</td>
<td>2</td>
<td>1:32</td>
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<td>V Sinus</td>
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<td>- + -</td>
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<td></td>
<td></td>
<td>26</td>
<td>± ± -</td>
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<td>27</td>
<td>- + +</td>
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<td>1:16</td>
<td>+</td>
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<td>34</td>
<td>± ± -</td>
<td>1</td>
<td>1:8</td>
<td>ND</td>
</tr>
</tbody>
</table>

*IAS - Turkeys placed in contact with the air sac principals; remaining groups listed are contacts to the sinus principals.

**Contact interval = Period of contact with the principals, e.g., 0 - 11 signifies turkeys placed in contact with the principals from zero to the 11th week after the principals were inoculated.

***T = tracheitis; AS = air sacculitis; P = pneumonia.

****Air sac lesions were graded from 0 to 3 depending on severity; score of 1+ or greater were active lesions.

*****ND - not done
Figure 2. Antibody response of turkeys placed in contact with the sinus principals at different times and held for varying intervals.

Group III contacts were turkeys two months of age. They were placed in contact with the principal group 19 weeks after the latter had been inoculated (Figure 2). At this time the GMT of the principal group was approximately 1:16 and nine birds had sinusitis. Although rales were evident in two of the principal birds the previous week, respiratory signs were not evident on the day the group III controls were introduced.

The group III controls remained serologically negative for a 15 week contact period. However, when killed on the 16th week one bird had a titer of 1:2 (Figure 2). Clinical signs were absent throughout the period of contact. Necropsy findings are presented in Table III.

Group IV controls were placed in contact with the principal group 24 weeks after the latter had been inoculated. (The group III controls, introduced five weeks earlier, were also in the same unit). The GMT of the principal group at this time was 1:8. Although sinusitis was still evident in nine of the birds, rales had not been evident for a period of five weeks prior to the introduction of the group IV controls. Clinical signs of rales and first detectable sign of antibody response were observed simultaneously seven weeks p.c. At necropsy four weeks later, the titer was still rising (Figure 2). Table III shows the necropsy findings.

Five turkeys in the sinus-principal group were killed at 36 weeks to determine the status of the group; one was killed earlier at 34 weeks since it was moribund (Table III). This left four turkeys in the principal group for further transmission studies. Contact groups V and VI, birds of four and nine months of age, respectively, were placed in the unit at 36 weeks. On the basis of the antibody response (Figure 2) and the necropsy findings (Table III) on these contacts, the sinus principals were still infectious between the 36 to 47 week period after inoculation. Furthermore, birds four months of age were just as susceptible as those nine months of age.

The final contact transmission study on the sinus principals was initiated at 56 weeks p.i. The group VII controls placed in the unit at this
TURKEYS EXPOSED TO MYCOPLASMA GALLISEPTICUM 585
time remained negative clinically and serologically for an 11 week contact period (Figure 2). To determine further the status of control group VII after this contact period, two of the five turkeys were inoculated via the sinus route with pathogenic Mycoplasma; they were found susceptible. The remaining three were necropsied and found free of gross lesions. At termination of the contact period (67 weeks p.i.) two of the four principals still exhibited sinusitis as a result of original inoculation. Tissues taken from the four birds yielded Mycoplasma from two sinus and one tracheal culture. These isolates were not pathogenic for normal turkeys.

DISCUSSION

The higher antibody response of turkeys inoculated by the sinus route as compared to those inoculated by the air sac route probably reflects the greater amount of tissue involvement in the sinus group. It appears that turkeys inoculated by the air sac route reacted to the agent primarily in that area. However, when the agent was inoculated into the sinus, there was not only a local tissue reaction at that site but also others in the trachea and air sacs. In this connection Adler et al. 8 (1962) found that turkeys with sinusitis as the only lesion, had a lower titer than those with extensive respiratory tract pathology.

Turkeys placed in contact with the sinus principals at the time the latter were inoculated responded with agglutinins at two weeks p.c.; those placed in contact with the air sac principals at the same interval did not respond until five weeks p.c. Further, the peak titer in the sinus contacts was much greater than in the air sac contacts (1:128 vs. 1:16) (Figure 1). This trend in response was confirmed in another trial. It appears that during the acute stage of disease, the additional dosage of infectious agent being released readily from the sinuses of the sinus principals could account for the shorter incubation period and higher antibody response in the contacts as compared to that of the air sac group.

Furthermore, while the air sac principals were no longer contagious 11 weeks after inoculation, the sinus principals were still contagious at 36 weeks. Both of the principal groups recovered from clinical signs of rales at approximately the same time (12 to 18 weeks p.i.). The only obvious difference between the two groups was the severe sinusitis manifested in the sinus principals which persisted to some degree for the entire 67 week study period. Thus, turkeys with lower respiratory infection apparently were able to transmit the agent by contact only as long as they were acutely distressed; on the other hand, the ability of turkeys with sinusitis to transmit over a prolonged period seemed to be governed by the persistence of the sinusitis, since transmission continued to occur long after the birds ceased to show rales.

In regards to the group II air sac contacts, it should be pointed out that a contact period beyond 11 weeks may have resulted in transmission since the principals still harbored the organisms in their air sacs when killed and examined two weeks after the group II contacts were removed. Nevertheless, the ready transmission from the sinus principals to their
controls during the same interval clearly shows the difference in spreading potential of the two groups.

As to transmission from the sinus principals to contacts, there appeared to be a drop in the dosage of the organism with clinical recovery in the turkeys. This was reflected in the longer incubation period, the lower level of antibody response, and the failure to obtain a sharp peak titer in the later periods of contact exposure (i.e., groups V, VI, and VII). However, in the group III controls, the younger age of the turkeys (two months) may have been a factor in the extended incubation period.\textsuperscript{13}

In most cases, antibody response, clinical signs, and necropsy findings were positively correlated. The first sign of antibody response either coincided with or preceded clinical signs by one or two weeks in both the principal and control groups. The culture results, however, were not entirely satisfactory. The isolations were generally poor even when the suspected tissues were first passaged through chicken embryos. It should be mentioned, however, that isolations from the sinus exudates were much more successful than those from the air sacs, even when the former were taken from chronically infected and the latter from acutely infected birds.

**SUMMARY**

1. The plate agglutination test was a valuable tool in following the clinical course of *Mycoplasma gallisepticum* infection in turkeys exposed either by inoculation or by contact.

2. A five-minute holding time of the serum-antigen reactants prior to reading gave satisfactory differentiation among sera of varying reactivity.

3. There was good correlation among antibody response, clinical signs, and pathological findings at necropsy.

4. Turkeys exposed by the sinus route responded with a much higher geometric mean titer at the peak of response than did those exposed by the air sac route (i.e., 1:256 vs. 1:45).

5. Turkeys exposed by the sinus route were capable of transmitting the agent to contacts much more rapidly during the acute stage of the infection, and over a longer period, than were those inoculated by the air sac route.

**REFERENCES**


Ectoparasitism by arthropods and diseases so transmitted constitute a formidable hazard to profitable livestock production. It is difficult to place dollar values on losses due to ectoparasites. However, in 1958 the U. S. Department of Agriculture estimated that, prior to the eradication program, losses from infestations by the screw-worm (Cochliomyia hominivorax (Coquerel)) exceeded $20 million annually in the Southeastern United States. Oglesby estimated that anaplasmosis, transmitted by biting flies and ticks, costs U. S. livestock producers between $34 and $35 million annually in morbidity and death losses. Although these estimates may seem high, they are specific examples of the magnitude of the ectoparasite problem.

A survey of the literature pertaining to arthropods of veterinary importance indicates that a great deal of research has been devoted to two principal lines: 1) Investigations on the biology and control of economic species, and 2) epizootiological studies on known and potential vectors of diseases. A comprehensive review of the many reports on these subjects is beyond the scope of this paper. However, a number of factors peculiar to certain species or groups of ectoparasites contribute to their importance as pests of livestock and vectors of diseases. The purpose of this paper is to discuss a few of the important groups of arthropod pests and their relationship to some of the diseases they transmit.

THE HORSE FLIES AND DEER FLIES

Members of the family Tabanidae, commonly referred to as tabanids, horse flies, and deer flies, are among the most important livestock pests. Approximately 500 species are known from the United States and Canada, and all but a few of these are avid blood suckers. Nearly all economic species are members of the genera Tabanus, Hybomitra, and Chrysops. In northern regions tabanids are active principally during June, July, and August; but in the Southern States the tabanid season extends from March or April through October or November. In extreme southern Florida, tabanids may be active throughout the year.

Tabanids are usually thought of as pasture or range parasites. Their breeding areas vary from aquatic or semiaquatic to terrestrial situations. The developmental period for most species is about one year, but some may require two years. In the Southern States a few species may produce
two generations a year. Adult habitats are usually associated with the breeding areas, although the females may fly several miles in search of a suitable host. The males, which do not suck blood, are rarely seen around livestock.

In general, the horse flies and deer flies are diurnal feeders, attacking livestock during the bright, warm hours of the day. However, several species feed principally at dawn and dusk, and a few species have been collected from livestock after dark. The severity of an outbreak of these flies can be appreciated only by actual observation. In Florida, I have seen cattle actually driven from pastures by the flies. During such attacks the animals stop grazing, bunch together, and constantly attempt to fight off their tormentors. Animals subjected to these high-density populations frequently become lethargic and finally stop fighting the flies, thus leaving themselves open to a heavier assault. Aside from the obvious amount of blood lost owing to the feeding of the flies, an often overlooked factor is the amount of blood that exudes from the feeding puncture after the engorged fly leaves. Horses and cattle may appear crimson over the necks, sides, flanks, and legs from this flow of blood. After several days of heavy attack, the animal's hair becomes matted with dried blood over the greatest part of its body.

The voracious appetites and their feeding habits enable tabanids to be efficient mechanical vectors of diseases. Some species habitually make a number of feeding punctures before feeding to satiety. If such flies are disturbed, they may transfer directly to other nearby hosts to continue their attacks. Species that usually feed to repletion during a single bite are frequently dislodged, and these flies may or may not return to the same host. I have observed a single specimen of *Tabanus americanus* Forester attack and feed on five animals during a period of 12 minutes before its blood meal was finally obtained.

The relatively large size of the mouthparts of Tabanidae is also a factor in their ability to transmit diseases since a large surface area will, of necessity, become contaminated with blood during feeding. It is easy to see that when a fly bites an animal with pathogenic agents in its blood and is disturbed, the next host will be subject to invasion of the disease agents contaminating the mouthparts of the fly.

The diseases transmitted by Tabanidae have been reviewed\(^1\) and a detailed discussion of the known species that transmit each disease will not be attempted here. The transmission of anthrax by tabanids has been demonstrated; however, the importance of the flies in the natural dissemination of the disease is not known. Transmissions of *Pasteurella tularensis*, the causal agent of tularemia, have been made from rabbit to rabbit by immediate transfer feedings of *Chrysops discalis* Williston. This deer fly appears to be the principal arthropod vector of tularemia to humans. *Chrysops* spp. are vectors of the African eye worm, *Loa loa* Guyot, and these flies are now considered to be the only vectors of this disease. At least five species of trypanosomes are transmitted by tabanids, and among these are some of the most serious diseases of livestock. Equine infectious anemia has also been experimentally transmitted by tabanids.
In the Central and Southern United States tabanids appear to be important vectors of bovine anaplasmosis. Experiments by Sanborn et al., Sanders, Lotze and Yiengst, and Howell et al. showed that these flies can transmit the disease, and heavy annual losses from anaplasmosis following infestations of tabanid flies provide further circumstantial evidence of their vector status. As far as is known, anaplasmosis is transmitted by tabanids in a purely mechanical manner. Although the feeding habits of the flies make them efficient vectors of this disease, the mechanical type of transmission enables us to provide a method of reducing the disease's incidence. Howell et al. found that the causal agent of anaplasmosis (Anaplasma marginale Theiler) remained viable on the mouthparts of a fly for only a short time—probably less than five minutes. Thus, if known infected or reactor animals could be eliminated or isolated from susceptible cattle, the disease would have little chance of transmission. Hoffman et al. demonstrated that tabanid populations can be reduced and anaplasmosis transmission minimized by daily sprays of synergized pyrethrum applied by a photoactuated walk-through-type sprayer.

THE MOSQUITOES

Mosquitoes have been intensively studied for their depredation of man; however, few people recognize their importance as livestock and poultry pests. About 150 species occur in the United States and Canada. Flight habits, food preferences, and climatological requirements vary widely; but all species require water for development of the immature stages. It is difficult to think of a collection of water that would not support the development of at least one species, and great swarms of these insects can be produced in extremely small quantities of water.

Species of a number of genera, such as Anopheles, Culiseta, Culex, and Mansonia, deposit their eggs directly on the water. The eggs hatch in a few days and the immature stages develop in two to three weeks to several months, depending on temperature and other climatological conditions. During the summer months some of these mosquitoes may produce a new generation every 15 to 30 days. However, other species may produce only a single generation a year. Species of the genera Aedes and Psorophora lay their eggs in situations that are damp or dry, but which will become flooded. Some species have only a single generation a year, but others may produce many generations or broods each year if alternate drying and flooding of the breeding places occur. Psorophora confinnis (L.) frequently becomes an important pest mosquito in the rice-growing areas of Arkansas, Louisiana, and Texas. Great swarms of this species occur shortly after the rice fields are flooded and livestock, poultry, and man may be severely attacked.

Sylvatic and pasture species are important from the standpoint of virus transmission. These species are in direct contact with birds which may serve as reservoirs for a number of encephalitic viruses, and in these ecological situations the avian-mosquito reservoir is kept intact. When they enter these habitats, man and other susceptible vertebrates are thus
open to infection. The importance of mosquitoes as vectors of virus diseases was pointed out by Reeves,16 who stated that "of approximately 125 viruses that infect vertebrates and are believed to be arthropod borne, circumstances of isolation and epidemiological associations would indicate that at least 100 are mosquito borne."

Mosquitoes are the only vectors of the malarias (*Plasmodium* spp.). *Anopheles* spp. are the only known vectors of *Plasmodium* infections in man, but the avian plasmodia may be transmitted by species of a number of other genera, including *Anopheles*.

Species of at least three genera of mosquitoes (*Aedes*, *Anopheles*, and *Culex*) have been reported to be vectors of *Dirofilaria immitis*, the heart worm of dogs and cats. The mosquito is a true intermediate host. The microfilaria develop in the mosquito and within five to 10 days the infective forms invade the mouthparts, where they are transmitted to the next host during the act of feeding.

**THE BLACK FLIES**

Flies of the family Simuliidae are small humpbacked gnats, commonly referred to as black flies and buffalo gnats. They are important pests of livestock, poultry, and man. Because of their small size, their attacks on livestock frequently go unnoticed unless the populations are of outbreak proportions. They attack horses, cattle, sheep, and goats, principally around the head; the ears, eyes, muzzle, and nostrils are favored feeding locations. Inspection of these areas may frequently reveal evidence of black fly feeding. Black flies attack their avian hosts about the head; the comb and wattle of domestic poultry are especially preferred feeding locations.

This group of insects breeds only in flowing water. Swift, shallow mountain streams are ideal habitats for many species; but a number of these same species may adapt to slow-moving, sluggish streams. A number of important southeastern species breed in sluggish streams and rivers. Eggs are laid on or in the water and after incubation periods of several days to several months, the eggs hatch. The small larvae are swept down-stream, where they attach to vegetation, sticks, stones, and other debris. Pupation usually takes place at the site of larval attachment. When the adult fly emerges from the pupal case, it rises to the water surface on a small bubble of air. The principal periods of adult activity are the spring and early summer months, but some species may be present from early spring until late fall. Under the proper climatological and ecological conditions, many species may produce a new generation in 30 to 40 days; however, others have only a single generation each year.

Black flies are vectors of bovine onchocerciasis20 and *Leucocytozoon* infections in poultry, waterfowl, and game birds.19,5,6 The transmission of some avian trypanosomes by black flies has also been demonstrated.7

Fallis et al.5 showed that the black fly is a necessary host in the transmission of *Leucocytozoon simondi* to ducks. Sexual development of the parasite occurs in the simulid vector. The developmental period is
variable, but active sporozoites were found in the stomach of a specimen of *Simulium rugglesi* Nicholson and Mickel 72 hours after the fly had fed on an infected host. Asexual development occurs in the avian host. The prepatent period usually requires 14 to 18 days. The parasite affects young ducklings more severely than older birds. Species of *Leucocyctosom* affecting turkeys and other avian hosts are believed to have similar life cycles in their simuliiid vectors.

**SAND FLIES**

These are very small (0.5 to 5.0 mm. in length) bloodsucking gnats of the family Ceratopogonidae. They are commonly referred to as "no-see-ums," "punkies," and "sand flies." Although the family contains other genera, the genus *Culicoides* appears to be of more importance from the veterinary aspect.

Breeding habits of the different species of *Culicoides* vary widely; however, in general, the larvae are aquatic or seminaquatic. The immature stages can be found in moist soil or mud of high organic content bordering fresh, brackish, or salt water. Some species develop in wet, decaying plant material, as well as in tree holes and sewage drain fields. Some species produce two or more generations each year but others may require one or two years to complete development.

The adult females are fierce biters, attacking man, livestock, poultry, and wild animals and birds. Owing to their small size, infestations of these gnats are rarely noticed on livestock and poultry; however, they can be readily observed if one is looking specifically for them.

*Culicoides* have been incriminated as vectors of *Hemoproteus* infections in wild and domestic ducks and in other birds. Sporogony of the parasite occurs in the *Culicoides* vector and schizogony occurs in the avian host. There is evidence that *Culicoides* may transmit some avian trypanosomes.

Du Toit first reported that *Culicoides* were capable of transmitting bluetongue virus in sheep. Work in the United States has demonstrated *Culicoides* as a vector of bluetongue.

**TICKS AND MITES**

Ticks are probably the most important single group of arthropods affecting livestock. Discounting the many diseases ticks may transmit, their parasitic activities alone cause great losses in livestock and poultry production each year. Taxonomically, the ticks are divided into two families. The hard ticks, family Ixodidae, are scutate or shield ticks characterized by anterior mouthparts or capitulum and a dorsal scutum which covers the anterior part of the body of female ticks and the entire dorsum of the body in male ticks. The soft ticks, family Argasidae, have ventral mouthparts, lack a scutum or shield, and have a leathery integument.

There are 16 species of soft ticks in the United States. The spinose ear tick, *Otobius megnini* (Duges), is a troublesome livestock pest in the
Southwestern United States. This species attacks the ears of all classes of livestock and a number of wild animals. It is not known to be a vector of disease. The fowl tick, *Argus persicus* (Oken), attacks most species of domestic fowls and several species of wild birds. It frequently becomes a serious pest in chicken houses and other poultry-producing installations. It has been demonstrated as a vector of avian spirochetosis and fowl piroplasmosis. Heavy infestations of *A. persicus* may cause fowl paralysis and death. A number of *Ornithodoros* spp. are known vectors of relapsing fever spirochetes.

There are 50 known species of hard ticks distributed throughout the United States. This does not include *Boophilus microplus* (Canestrini), which is occasionally found in the buffer zone along the Texas-Mexico border. Ecologically the hard ticks are, with few exceptions, range or pasture parasites. Their natural habitat brings them into direct contact with most classes of livestock, as well as with wild animals and birds, which, on occasion, may serve as reservoirs of diseases.

The life cycle has four stages—egg, larva (or "seed tick"), nymph, and adult. Engorged females drop from the host, and begin oviposition within a few days. The hard ticks deposit the eggs in masses ranging in size from less than 1000 to 18,000 eggs. The egg production for most species averages between 3000 and 5000. When oviposition is completed, the female dies. Eggs hatch in about 30 days, depending on environmental conditions; and the small hexapod larvae seek a suitable host. Some species require three hosts for development, some require two, and others only one. After feeding to repletion at each stage, three-host species drop from the animal to molt. Two-host ticks spend the larval and nymphal stages on the same host, then drop and molt; the adult seeks a second and final host. One-host species board the host as larvae and pass all molts on the same animal. With a few exceptions, three-host ticks usually produce one generation a year. However, some of the one-host ticks may develop from egg to egg in 70 to 80 days.

Most species of ticks are not host specific, but it is evident that some species have distinct host preferences in their different stages. As adults, three-host ticks such as *Dermacentor venustus* Banks (=*andersoni Stiles*) and *D. variabilis* Say readily feed on all classes of livestock and man; however, their immature stages feed almost exclusively on small mammals such as rodents. In the United States all stages of the brown dog tick (*Rhipicephalus sanguineus* (Latreille)) are pests only of dogs; records from other hosts are rare. However, Hoogetraal\(^{10}\) listed a wide host range for this species in Africa but indicated that dogs and their relatives are the preferred hosts. All stages of the long star tick (*Amblyomma americanum* (L.)), readily attack man and livestock, although the immature stages are frequently found on small mammals and occasionally on ground-nesting birds. The tropical horse tick (*Dermacentor nitens* Neumann) is a one-host tick that principally attacks equines. However, there are records of this species on cattle, sheep and goats.

Ticks are known vectors of protozoal, bacterial, rickettsial, and viral diseases. The number of diseases transmitted to livestock by ticks exceeds
that of any other group of arthropods. Neitz lists 25 piroplasms transmitted by ticks, and most of these parasites have one or more developmental stages in the vector; thus, the tick vector becomes a necessary link in the epizootological chain. In the Western United States, bovine anaplasmosis is believed to be transmitted principally by ticks. Bacterial pathogens such as *Pasteurella tularensis* are capable of undergoing multiplication in *Dermacentor venustus*, and several species of ixodid ticks have been incriminated as vectors of relapsing fever spirochetes. A number of rickettsial agents are exclusively tick borne. Heartwater, caused by *Cowdria ruminantium*, is an important African disease affecting cattle, sheep, and goats. It is transmitted by the "bont" tick, *Amblyomma hebraeum* Koch. In the United States the spotted fever rickettsia is transmitted by several species of *Dermacentor* and *Amblyomma*. At present, our knowledge is very limited regarding the transmission to livestock of tick-borne viruses. Louping ill and Nairobi sheep disease, both of which affect sheep and goats, are known to be transmitted intrastadially by *Rhipicephalus appendiculatus* Neumann. Experimental transmission by ticks of encephalitic viruses has been shown, but the importance of ticks in the natural transmission of these viruses is not known.

Only a few of the many known species of mites are parasitic on livestock; however, these few are extremely important. The more troublesome species of mites affecting livestock belong to the genera *Sarcoptes, Psoroptes, Chorioptes*, and *Demodex*. All species produce the condition referred to as mange. However, the term "mange" is sometimes applied only to infestations of *Sarcoptes* and *Demodex*, whereas the term "scab" is applied to infestations of *Psoroptes* and *Chorioptes*.

The mange, or itch, mites, are obligate parasites inhabiting the skin of mammals. Most species are regarded as varieties of *Sarcoptes scabiei*; i.e., *Sarcoptes scabiei* var. *equi* Gerlach is the causal agent of barn itch, mange, or scabies in horses. These are burrowing mites that make tortuous tunnels in the epidermis. Eggs are laid in the tunnels; the immature stages hatch and begin new tunnels, thus rapidly increasing the area of infection. A new generation may be produced in about two weeks. The scab mites (*Psoroptes* spp.) do not burrow, but live at the base of the hairs. Their bites produce inflammation followed by a serous exudate, which forms a scab over the infected area. Eggs are laid in the scabs. Because of the loose condition of the scabs, this form of acarasis is easily distributed from animal to animal by contact or by rubbing against stalls, fences, and similar objects. The sheep scab mite, *Psoroptes equi* var. *ovis* (Hering) can develop from egg to egg in nine days.

*Chorioptes bovis* var. *bovis* Gerlach produces lesions similar to those of *Psoroptes*, but the colonies are usually smaller and show less tendency to rapid spread. These mites are frequently restricted to the tail and limbs of cattle and the parasitic condition is often referred to as "tail mange." Demodectic mange is most common in dogs; however, *Demodex follicularum* var. *bovis* Stiles produces nodular lesions in cattle that seriously affect the value of the hides.
ARTHROPOD VECTORS

ARTHROPOD VECTORS OF HELMINTHS

Arthropods are utilized as intermediate hosts by many species of worms parasitic in livestock and poultry. Insects demonstrated to serve as intermediate hosts to helminths include a number of beetles representing at least five families, as well as cockroaches, grasshoppers, biting lice, dragonflies, fleas, house flies, stable flies, mosquitoes, and deer flies. Arachnids known to serve as intermediate hosts for helminths include several species of orbatic mites, frequently referred to as beetle mites. With the exception of the blood-sucking insects, which transmit filarial worm, these insects accidentally or habitually inhabit excrement in one or more stages of their life cycles.

Insect-borne worms of livestock and poultry include representatives of the four major groups of helminths, i.e., nematodes, cestodes, acanthocephalids, and trematodes. Those which may be transmitted by arthropods have the eggs or larvae of the worms occurring in the feces of the principal host.

Several species of dung beetles and tumblebugs serve as intermediate hosts for stomach and gullet worms of swine, sheep, cattle, and poultry. The beetle ingests the worm eggs and development to infective forms occurs in the beetle’s body. Transmission to the principal host occurs when the beetle is inadvertently eaten.

REFERENCES

A STANDAR D METHOD USING ANIMAL INOCULATION
FOR THE DETECTION OF HOG CHOLERA VIRUS


A. Obtaining samples from suspected diseased hogs

A major requirement in testing of suspected material is to obtain samples suitable for test. Material must be taken from freshly killed hogs or blood can be taken from sick animals. These must be obtained in clean sterile containers without contamination with outside bacteria or with chemicals (formalin, alcohol, etc.), plastic bags make suitable containers for tissues. Pieces of gut or other contaminated organs must not be placed in containers with blood, spleen, liver, lung and heart, or other organs that are normally free of bacteria. The spleen and blood are among the best for test. The entire spleen and/or 50-75 ml. of blood are obtained. These are well identified, chilled at once and conveyed to the laboratory under refrigeration. Means that allow contamination or temperatures that allow growth of bacteria will not suffice. Tissues contaminated with chemicals or bacteria are usually worthless.

B. Preparation of material from suspects for injection

Because the virus of hog cholera has an affinity for blood cells, it is desirable that the blood be defibrinated when it is to be employed in a test for the presence of hog cholera virus. The serum is employed in case the blood has clotted. In either case, the test dose is 5 ml. injected subcutaneously or intramuscularly.

Where an extract from the spleen or other internal organ is employed, the tissue is thoroughly ground with a mortar and pestle (or other grinding apparatus) with or without sterile abrasive, in diluent (sterile physiological salt solution, Earle's or Hank's fluid, etc.) one part tissue to four parts diluent. Should the mixture or emulsion contain pieces that do not settle readily or if it is suspected that bacteria are present, the suspension is centrifuged at 1000 x g. for 10 minutes. The dose of the supernate is 5 ml. as above. Cultures for the demonstration of the bacteria are made on artificial medium. Materials for injection (either blood or tissue extracts) are not filtered unless there is reason to suspect they contain bacteria that would interfere with the test.

C. Animals employed in the test

Specific pathogen free (SPF) pigs of 35-60 lbs. wt. are recommended. If pigs other than S.P.F. are employed, it is recommended that they be litter mates except that the immune control need not be a litter mate. (See below). Another choice of test pigs is from well-known herds whose health
status is well-established through long knowledge and study of such herds. Naturally, test pigs should come from herds where there has been no cholera and no vaccination. Five animals are employed as follows:

1. One immune pig is injected with material from the suspect.
2. One susceptible pig is injected with 15 ml. of known (tested) hog cholera antiserum and with the material from the suspect.
3. Two susceptible pigs are injected with material from the suspect.
4. One susceptible pig is uninjected.

The uninoculated susceptible control pig (#4 above) is kept in strict isolation by itself - the other four pigs may be maintained together throughout the test, but must be separated in case of excessive fighting.

D. Exposure to virulent virus

Should any of the pigs in No. 2 or 3 or 4 above remain healthy, they are injected either subcutaneously or intramuscularly with 2 ml. of known" (see below) hog cholera virus 28 days after the original exposure.

E. Interpretation of Results

If the pig in No. 1 remains healthy, this is taken to mean that the suspect material contains nothing injurious to a cholera immune control - if he sickens, the material contains one or more disease agents other than hog cholera virus.

If the pig in No. 2 remains healthy, this is taken to mean that the suspect material contains nothing injurious to pigs with the possible exception of hog cholera virus. If the pig in No. 2 does not sicken and the pigs in No. 3 do sicken, this is taken to mean that virulent cholera virus is present in the suspect material - if the pigs in both No. 2 and No. 3 sicken, this is suggestive that a variant hog cholera virus is present.

The pig in No. 4 is the susceptible control - he should sicken when exposed to known virulent virus. Such sickness is taken to mean that pigs in No. 2 and No. 3 were susceptible.

Should the pigs in No. 3 survive both the initial exposure to the suspect material and the final exposure to known virulent virus, this would suggest the presence of an antigen in the suspect material that stimulated immunity to hog cholera.

A known virulent hog cholera test virus is one that has a known infectivity titer but has no demonstrable variant characteristics. Such facts regarding a test virus are established by tests on pigs.

A known hog cholera antiserum is one that has been tested insofar as possible (with regard to protection, etc.).

Once data have been obtained on a strain of hog cholera virus or a batch of hog cholera antiserum so that it is "known," stocks of such virus and serum should be maintained for testing suspect cases.

Presently, it appears that fluorescent antibody technique for the demonstration of virus in the blood or tissues of test pigs seven days post-exposure is an adequate means of diagnosis. Confirmation awaits additional study.
FLUORESCENT ANTIBODY FOR DIAGNOSIS OF HOG CHOLERA


Veterinary clinicians and pathologists have long recognized the difficulties inherent in establishing an accurate and positive diagnosis of hog cholera (HC) when an outbreak of disease occurs. The variability of signs and symptoms has frequently confused even the more experienced swine disease investigators. The variability of pathological changes has stimulated the search for a test or procedure which would provide a rapid accurate diagnosis. This search has been going on since the recognition of the disease as an entity. Many field and laboratory procedures have been developed, but each has certain deficiencies and has thus failed to provide a completely satisfactory method of diagnosis.

Consideration of a nationwide cooperative Hog Cholera Eradication Program has increased the need for an accurate means of diagnosing the disease. It is particularly essential if indemnities are to be paid for the destruction of infected hogs under the eradication program.

An ideal test would be one that could be applied easily and completed in a few minutes by a veterinarian in the field. Such a test should also be inexpensive and applicable on a large scale. At the present such a test does not exist but the fluorescent antibody (FA) test as described by Stair et al.9 embodies rapidity, accuracy, and applicability on a large scale at a moderate cost.

MATERIALS AND METHODS

All pigs used in the propagation of hyperimmunizing HC virus and for the production of specific HC antiserum were hysterectomy-derived, colostrum-deprived, littermate pigs reared in isolation.11,12

Hyperimmunizing HC virus antigens were obtained as one percent citrated blood plasma from pigs infected five days previously with one of three strains of virulent HC virus. The immunization procedures were as follows: 1) A vaccination dose of high passage lapinized hog cholera virus was given at 10 weeks of age; 2) A challenge dose (two ml.) of virulent virus, strain V1, was given subcutaneously postvaccination day seven; 3) One hundred fifty ml. of virulent virus, strain V2, was administered intraperitoneally postvaccination day 14; 4) One hundred twenty-five ml. of mixed equal volumes of virulent viruses, strains V1 and V3 were inoculated intraperitoneally on postvaccination day 24; 5) These serum producing pigs were exsanguinated two weeks after the last hyperimmunization dose of virus and the serums were harvested.

The antiserum was placed in a cellulose dialysis bag and dialyzed for 24 hours against ammonium sulfate so that a final concentration of 1.39 M ammonium sulfate was present in the antiserum. The precipitated globulins were collected by centrifugation and taken up in solution with a
minimum of 0.05 carbonate-bicarbonate buffered saline pH 9.0. The solution of globulins thus obtained was dialyzed against the same carbonate buffer until it was free of sulfate and a pH of 9.0 was reached.

Protein concentration was determined by the method of Lowry et al. and diluted with the carbonate-bicarbonate buffer to a final concentration of two percent. Crystalline fluorescein isothiocyanate (FITC) was added by dialysis with stirring to the globulin solution at a rate of 24 mg of FITC per gram of globulin. This conjugation was allowed to proceed for 24 hours at 4°C.

The conjugate was then dialyzed versus 0.01 M sodium phosphate buffer pH 7.5 and AG 2 X 4 ion exchange resin until the solution approximated the ionic strength and pH of the buffer and the free fluorescein had been removed.

The conjugate was absorbed to a diethylaminoethyl cellulose column 2.3 cm in diameter, 16 cm in length, and washed with five column volumes of the 0.01 M sodium phosphate buffer. A gradient was applied by using a 250 ml. mixing chamber and running 1.0 M sodium chloride in 0.01 M sodium phosphate buffer pH 7.5 into the mixing chamber (Figure 1).

Figure 1. A schematic representation of the apparatus used for diethylaminoethyl gradient column chromatography.
Fractions thus obtained were evaluated at 280 and 495 millimicrons on a spectrophotometer and the fluorescein to protein ratios computed. Fractions having a fluorescein to protein of 3.8 to $5.4 \times 10^3$ were pooled and dialyzed versus 0.001 M phosphate buffered saline pH 7.5. The conjugate was then ready for use. For more detailed information the reader is referred to Goldstein et al.\textsuperscript{3,4}

Seventy-two hysterectomy-derived, colostrum-deprived pigs maintained in isolation were inoculated with hog cholera virus (Table I).

**TABLE I**

Intensity of Fluorescent Antibody Reaction in Swine Tissues Infected with Different Hog Cholera Viruses

<table>
<thead>
<tr>
<th>Hog Cholera Antigen*</th>
<th>No. pigs Inoculated</th>
<th>Intensity of Fluorescence**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virulent viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_1$</td>
<td>12</td>
<td>++</td>
</tr>
<tr>
<td>$V_2$</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>$V_3$</td>
<td>2</td>
<td>++</td>
</tr>
<tr>
<td>$V_4$</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td><strong>Modified viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine: $P_1$</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Rabbit: $R_1$</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>$R_2$</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td><strong>Tissue culture viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$TC_1$ (virulent)</td>
<td>35</td>
<td>++</td>
</tr>
<tr>
<td>$TC_2$ (attenuated)</td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>

*Viral antigens in frozen sections from organs of HC infected pigs.

**FA composed of globulins of pigs injected with $R_1$ and $V_2$ HC viruses and hyperimmunized with $V_2$ and a $V_1V_3$ combination of HC viruses.

++Intense, +Acceptable, —None.

Fifty-eight of these pigs received five different strains of virulent virus by various inoculation routes. Three of these virulent virus strains were those used as antigens for the production of hyperimmune serum. The other two virulent viruses used were the Ames strain and a virulent tissue culture strain which originated in this laboratory. The remaining 14 pigs were inoculated with four attenuated vaccine strains of HC virus. Freezing and cutting of infected tissues was carried out as described by Coons and Kaplan.\textsuperscript{2} Sections were fixed by drying. All solutions used in the staining process were filtered immediately prior to use through a 0.45 micron millipore filter. The staining procedure was as follows: 1) The tissue was flooded with the labelled globulin; 2) Two to three drops of guinea pig complement were added and mixed in by rotation of the slide; 3) Slides were incubated for 30 minutes at 37 C in a humid chamber; 4) Slides were washed with at least three changes of 0.01 M phosphate buffered saline pH 7.5 over a period of 30 minutes; and 5) Coverslips were mounted with carbonate-bicarbonate buffered glycerol pH 8.0. Stained
tissues were evaluated microscopically on a microscope equipped with ultraviolet and incandescent light sources and appropriate filter systems.

RESULTS

Tissues from 58 susceptible pigs inoculated experimentally with five different strains of virulent HC virus gave specific fluorescence when they were evaluated by the fluorescent antibody technique. Hysterectomy-derived pigs which were either uninoculated, experimentally inoculated with pseudorabies, virus pneumonia of pigs, exudative epidermitis, or swine influenza served as a source of control tissues. No specific fluorescence was observed in tissues from any of these pigs. When tissues from infected pigs were treated with unlabelled anti-HC globulin prior to

Figure 2. Parotid salivary gland of pig inoculated seven days previously with virulent HC virus. Fluorescent staining of acini, not all acini are involved. 500 X.
application of the labelled globulin the staining reaction was completely inhibited. Fluorescence was observed from the third to the fourteenth postinoculation day. Tissues which gave the best staining reaction were the parotid salivary glands (Figure 2). Consistent staining results were observed with spleen and lymph node tissues, but these tissues did not stain with the same degree of brilliance and differentiation as the acini of the parotid salivary glands. Spleen and lymph nodes also required more detailed evaluation since both tissues contain cells which react nonspecifically with fluorescent antibody. These cells were prominent when viewed with either incandescent or ultraviolet light. The submaxillary salivary gland did not stain as consistently as the parotid salivary gland and when staining was observed it had a particulate distribution within the cytoplasm.

Figure 3. Cross section of collecting tubules in the kidney of a pig inoculated eight days previously with virulent HC virus. Epithelial lining cells are the only structures containing fluorescent material. Background fluorescence is a blue-gray autofluorescence. 500 X.
rather than the diffuse pattern which was observed in other tissues. The kidney stained irregularly with most staining reactions apparently occurring in pigs which had been inoculated seven to fourteen days prior to death. Staining reactions were observed in the tubular epithelial cells of the kidney with no involvement of the glomeruli (Figure 3). When staining reactions were observed in the brain they were confined to the blood vessels and perivascular structures. Lung tissue was not consistently stained but when staining did occur it was found in the epithelial lining of the bronchi and bronchioles. The subepithelial mucous glands were also stained in these sections. Fourteen pigs were inoculated with four different strains of attenuated viruses. Specific fluorescence was observed in the tissues of only one of these pigs. This pig became ill at nine days postinoculation and died 11 days later. Gross and histopathologic lesions were typical of hog cholera.

DISCUSSION

The fluorescent antibody procedures described in this article have most of the desirable characteristics enumerated in the introduction. When compared with the time-honored method of inoculating susceptible pigs with material from suspected pigs the FA procedure provides several advantages. The elapsed time between collection of tissues and a final decision is much shorter with the FA technique. Materials and equipment needed to conduct the FA test can be prepared in advance and will thus be available when needed. Supplies of susceptible pigs are usually limited and expensive. Materials used in the FA procedure can be standardized and permit a more uniform basis for testing than can susceptible pigs. Diagnosis of hog cholera by inoculation of susceptible pigs is again based on clinical signs and tissue changes, some of which may be altered if more than one etiologic agent is present in the inoculum or is latent in the test pigs.

The use of leucocyte counts, as suggested by Lewis and Shope, provides an indication of a viremia if the blood sample is collected during a period of leucopenia. The value of this procedure may be reduced if other viral or bacterial agents are present in the herd. Such a laboratory procedure is certainly of value in providing information to be combined with clinical and pathologic changes in establishing a diagnosis, but cannot be depended on to permit a definitive diagnosis in itself. The amylase and hemolytic tests of Taylor have not been reliable enough for specific laboratory diagnosis.

Inclusion bodies have been described by Boynton et al. in the mucosa of the gall bladder and by Urman et al. in reticulo-endothelial tissue. Preparation and examination of tissues for these bodies requires time and in some individuals they are not observed. Inclusion bodies require a period of three to five days to form and may disappear from tissue within ten to twelve days after infection first occurs. Finding such inclusions on which to base a diagnosis would require tissues from animals in this stage
of infection. FA has revealed infections three days after experimental inoculation and has given positive results for 14 days. The finding of inclusion bodies provides a definite diagnosis, but the margin of error is too great for use in an eradication program.

Histological examination of the brain may provide significant information. However, the lesions are not necessarily pathognomonic for hog cholera, since other diseases produce similar encephalitic changes.

Laboratory tests including the resin test described by Segre* in 1957 and the hemagglutination test of Pilchard and Segre7 present difficulties in the preparation and preservation of reagents needed to conduct these tests. Preparation of these reagents has been accomplished by the originators, but have presented difficulties to workers in other laboratories. Preparation of FA conjugates has been carried out in many laboratories and for numerous etiologic agents. Hog cholera FA conjugates have been stored under refrigeration for nine months without loss of activity. The FA test requires a union between fluorescent labeled antibody and the antigen in the tissue with illuminations under suitable light. Seventy-two experimentally infected pigs which furnished tissues for examination were reported under previous sections of this paper. Visualization of tissue antigen was successful in 59 of these pigs. The remaining 13 animals had been inoculated with attenuated viruses, and at the time of tissue collection were not exhibiting clinical signs of hog cholera. They apparently had insufficient antigens in their tissues to unite with the conjugate. None of the attenuated virus strains were employed in production of the hyperimmune serum used in preparation of the fluorescent conjugate. A specific conjugate prepared for such virus strains is currently being made. Such a conjugate may either be incorporated in a heterologous preparation or used separately. Tissues collected from twenty-one pigs submitted to the Diagnostic Laboratory have been examined by the FA techniques described. FA results were positive in six cases. The remaining 15 were negative on FA and subsequently other laboratory procedures indicated the presence of disease entities other than hog cholera virus. The six positive diagnoses were confirmed by bacteriological, hematological, and histopathologic procedures.

SUMMARY

A procedure is described for preparing a fluorescent antibody (FA) conjugate which has been successful in demonstrating antigens in tissues from hog cholera infected hogs. Hog cholera virus antigens were found most consistently in parotid salivary glands and have been observed in submaxillary salivary glands, lymph nodes, spleen, kidneys, lung, and brain. Work is in progress at the University of Nebraska to improve and simplify the techniques for diagnosis of hog cholera through use of FA.
REFERENCES


SIXTY-EIGHTH
ANNUAL MEETING
October 19 to 23, 1964
PEABODY HOTEL
Memphis, Tennessee