Proceedings
SIXTY-FOURTH
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

DANIEL BOONE HOTEL
Charleston, West Virginia
October 18, 19, 20, 21, 1960
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R. A. Hendershott, Trenton, New Jersey
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<tr>
<td>1. Sept. 27-28, 1897†</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Texas</td>
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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>Wichita, Kansas</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
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<td>11. Sept. 16-17, 1907</td>
<td>Washington, D. C.</td>
<td>*Dr. Charles G. Lamb, Colorado</td>
<td>*Dr. C. E. Cotton, St. Paul, Minn.</td>
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<td>18. Feb. 16-17-18, 1914</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. J. Gibson, Des Moines, Iowa</td>
<td>*Mr. J. F. Ferguson, Chicago, III.</td>
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<td>33. Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
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<td>*Mr. J. F. Eisenman, Washington, D. C.</td>
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<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
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<td>Atlantic City, N. J.</td>
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<td>58.</td>
<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
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<td>Nov. 28-29-30, 1956</td>
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<td>62.</td>
<td>Nov. 4-5-6-7, 1958</td>
<td>Miami Beach, Florida</td>
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Dr. T. C. Green, Charleston, W. Va. |
Dr. H. F. Wilkins, Helena, Montana |
Dr. A. L. Brucener, Baltimore, Md. |
Dr. G. H. Good, Cheyenne, Wyoming |
Dr. John G. Milligan, Montgomery, Alabama |
Mr. F. G. Milligan, Montgomery, Alabama |
Mr. James R. Hay, Chicago, Ill. |

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

HON. C. H. UNDERWOOD

Governor of West Virginia

Good morning Representatives and Delegates to this Sixty-fourth Annual Meeting of the United States Livestock Sanitary Association.

I have been here just long enough to hear about half of the preceding address, and my chest is already hurting.

You see, at this time of the year, Commissioner Johnson and I and others running for office get very sympathetic to the feelings of our people.

We try to lend ourselves to their situations at first hand, and this was good prey this morning. I don’t know what I am going to do when I leave here to get rid of it, but I will try not to shake hands with any monkeys on the way back.

It is a great pleasure for West Virginia to have the privilege of entertaining your annual meeting. It was my good pleasure, I believe two years ago, to join with the officials of the Department of Agriculture here to extend an invitation for your meeting to come to West Virginia. At that time, we thought you would enjoy seeing the Greenbrier Hotel, but we are just as delighted to have you here in the Capital City of West Virginia.

I hope and I am sure from a casual examination of your program that your meeting will be very interesting. It certainly indicates that your organization is in step with the trend of our times. As a layman in your field, I am much impressed and delighted to see the heavy emphasis that you are placing on research. It seems to me that in this country, we need to emphasize more and more research in fields other than strictly missiles and scientific approaches leading in that direction. We need to consider very much in the fields of human problems and the problems of our animal world, wherever they may be related.

I don’t know what you may have heard about West Virginia prior to your arrival here. I assume that your impression of this state may depend upon which of the journalistic hucksters you may have followed in the recent months. When I was born and reared on a farm in Tyler County, we used to look forward to the huckster coming once a week to pick up the eggs, cream, and so forth, and to buy the feed and other things. I have had to deal with other hucksters in other months. They come and I am not sure what they leave. I think that would be a good subject for research.

Only yesterday I visited in Mercer County, West Virginia, and I saw what is one of the most modern high schools—I dare say you won’t find a more modern building in the country, built by the people of that county who were going to finance it out of extra levies, bond issues, landscaped along the setting of the mountains. I am campaigning in a helicopter and when we
moved into that football field, the pilot said, "This looks like going into LaGuardia, compared to what we have been in."

The nation saw Mercer reported by the famous newsman, David Brinkley, last April or May—saw Mercer County pictured to the nation as a decrepit area of abject poverty, of backward people, of falling-down buildings, and it was in this same county when Mr. Brinkley arrived, he went to the Superintendent of Schools and said, "I want to see your worst school building in the county."

The Superintendent is a proud man. He has the finest schools in West Virginia. He said, "I will take you to the bad places and then I will show you some good ones."

Brinkley replied, "There is no news in a good school. I can find those anywhere."

We are a little irritated by these hucksters who run over us like rabbits on a grass patch, and they leave about the same thing that the grasshopper leaves when they go back.

We do have problems in West Virginia, and I suspect some problems that people from your organization will help us solve in the years ahead. We have also many fine things in this state. You are in the state represented by the thirty-fifth star in the flag, the only state in the country born without the consent of the mother state. You will recall when Virginia seceded from the Union in the Civil War, that we, in turn, seceded from them.

Abraham Lincoln blessed this secession inasmuch as it was for the cause he was supporting.

We seceded from Virginia and then we immediately proceeded to have a convention and adopt for the most part of the constitution of the State of Virginia, with few changes.

Comparatively speaking, this is one of the younger states, only 15 others younger than we. I would like for you to know that we look upon ourselves as a young state, much as a young person looks upon himself and the life ahead. We are blessed with a tremendous deposit of unmeasured and undetermined natural resources. We have a mountainous terrain that is not duplicated anywhere in the country. No other state in the country has a greater percentage of its land surface in mountain terrain than West Virginia. This has created problems in the past, but moving into a missile age, it may be a solution to the problem not only for ourselves, but other people who hover around us in other states.

We look on it as a state with a great future. It is not as congested as the area surrounding us for five hundred miles. I think it is a state which has a future in the field of research, has room for expansion, has a great desire to encourage scientific research in many fields, and we are developing in our state university one of the finest medical centers in the country, and under construction now is a new agricultural building which will lend its medley to research, and just across the way, almost within sight, is the research laboratory of the United States Bureau of Medicine. We hope to join in
with medical research and other things and move West Virginia into the fore-front as a leader in research.

It is a pleasure to have you here. You honor us with your presence. If there is anything I or my office can do to make your stay helpful, we will gladly assist you.

I know my Secretary of Agriculture will speak for the people of West Virginia. I speak as Governor of the people of the state saying that we are grateful to have you here. I must apologize that it will be necessary for me to leave almost immediately because of other commitments. I hope you enjoy your stay in Charleston, West Virginia.
WELCOME ADDRESS

HON. J. T. JOHNSON, Secretary of Agriculture

Charleston, West Virginia

Mr. Johnson: Mr. Chairman, Officers and Members of the United States Livestock Sanitary Association, the Governor has done a fine job in telling you about our state. You know, he is running on the Republican ticket and I am running on the Democratic ticket, and he likes to get all of our Democrat votes he can. I don’t blame him for that, but in wandering around in Tyler County, I heard a story on him. I don’t know whether he has left or not, but, anyway, when he was in the second grade, he went to school late one morning. The teacher said, “Cecil, did you stop at the Principal’s Office and get your permit to come in late?”

“No.”

She said, “Well, now, just why were you late?”

He said, “Teacher, I had to turn two cows in with the bull.”

She said, “Well, can’t your father do that?”

He said, “No. He is not registered. The bull is.”

To get down to more serious part of my talk, it is quite an honor and a real pleasure to welcome you folks to Charleston, West Virginia, our capital city. About 75 percent of our gross income in West Virginia comes from livestock. Therefore, our farmers are very, very much interested in your program and your work that you are doing here today. I know that your annual conference here will mean much to the farmers of our entire nation. Much depends on you folks. We welcome you to our mountain state and sincerely hope that you will return soon, either to go home or enjoy your visit here in West Virginia.

West Virginia has led all states in the production of bituminous coal since 1931. We have a supply left, mining at the present rate, to last over four hundred years in the future. Our state ranks second in the production of glass. Some of the finest glass in the world is made here in West Virginia and is shipped to practically all parts of the world.

We are third in the value of gas produced in the nation. We are third or fourth in the value of minerals produced.

We have 16 state parks comprising a million and a half acres devoted entirely to recreation. We also have two or three National Forests.

There are many historical spots in our state of interest to tourists. If you want to spend a vacation in luxury, just stop over at the Greenbrier Hotel at White Sulphur Springs in Greenbrier County. It is one of the finest hotels in the nation. They have every facility there for your enjoyment.
Our Western States have the highest mountains, biggest rocks, and deepest valley, but West Virginia has more natural beauty than any state in the nation. We are so close to so many people, more than 50 percent of our people are within five hundred miles of West Virginia, or a day’s drive by your car.

We want you all to come back, visit with us and bring your friends when you come.

West Virginia, land of beauty, land of birds and friendly flowers. We invite you to visit with us among these friendly hills of ours.
Honorable Governor Underwood and Honorable J. T. Johnson, Commissioner of Agriculture: We who are here from other states thank you for your cordial welcome and warm hospitality we have already experienced here in Charleston. It is a pleasure to visit your state which is so rich in the early history of our country. To us from the West, where ample water is a never-ending problem, your verdant forests and well watered plant life creates envy. I note that your state has the highest mean altitude of any state east of the Mississippi River (1500 feet), but even though you do practice some perpendicular farming, I doubt if your slopes are as steep as ours in Idaho where the cattle occasionally do actually fall out of the pastures. We of the West, who bask in the tales of cattle drives and Indian Wars, often forget that you folks here in West Virginia were making large cattle drives over the James River and Kanawha Turnpike to Eastern markets before the end of the 18th century, and that while your men were fighting in the Continental forces during the Revolutionary wars while others at the same time were holding off the British-led Indian attacks along the Ohio River Valley with its numerous forts.

We do well remember that your state has a wealth of natural resources, coal, oil, gas and that your industries in the 18th and 19th centuries pioneered in the industrial revolution that helped the Western pioneers to settle and develop the lands west of the Mississippi River. From the days of Daniel Boone, one of your early important citizens of Kanawha Valley, by the way, my son has one of his fur caps, your people and industrial products were an important asset in the growth of our country and it is very evident today, as we see the large chemical plants in this city, that you are still forging ahead in industry.

We also note that you have not lagged behind in agriculture. May I personally thank you for the annual shipment of mistletoe from Scott’s Mountain bringing us joy at Christmas time and the fine Buckwheat you export for our pancakes. Your Department of Agriculture is to be complimented on your state being the 22nd state to accomplish Modified Certified Brucellosis status on October 9, last year, and for your becoming the thirteenth Modified Accredited TB State in December of 1933, and it is good to note that your salesyards and stockyards are cooperating 100 percent in the very important Market Cattle Testing Program.

It seems to me that everything is in fine shape here, your industry is booming, your agriculture and livestock are healthy. Commissioner Johnson, we like your state and again thank you for this invitation to be here and hope we shall be welcome to come again. Thank you.
GREETINGS FROM THE AMERICAN VETERINARY MEDICAL ASSOCIATION

Dr. E. E. Leasure

Manhattan, Kansas

President Hay, Members of the United States Livestock Sanitary Association, Guests, Friends: I do appreciate having been invited to address you this morning as a representative of and on behalf of the American Veterinary Medical Association, I bring you greetings and best wishes for a very very successful convention.

The American Veterinary Medical Association considers the United States Livestock Sanitary Association as one of the allied professional groups as an important structure in the over all picture of organized veterinary medicine. American Veterinary Medical Association stands ready, and willing to lend assistance to the United States Livestock Sanitary Association at any time. Should there be any way to improve its relationship to this organization, please do not hesitate to call on its Governing Board.

The American Veterinary Medical Association is engaged in a broad range of activities, designed especially for the improvement of veterinary services to the American public, and for the improvement of veterinary science and medicine.

I should like to mention a few of these activities. Through its Council on Education, the American Veterinary Medical Association is the accrediting body for veterinary schools of medicine throughout the United States and Canada and in this respect has been an important force for improving the over all process of these institutions.

Criteria for acceptable schools of veterinary medicine was set up over 40 years ago, and veterinary schools readily accepted these requirements.

Veterinary medical education, services and research, have made outstanding gains in the United States and Canada in the past 40 years, and in these, many of you have played an important role and have profited in these gains. The profession has grown rapidly because of its improved education and consequently because of its ability to provide improved services needed by the public. With the expansion of our population, an ever-increasing burden will be placed on agriculture to produce more food and animal food products for consumption.

This will affect veterinary services and there will be more demand for veterinarians in the future to take care of this increasing animal population so necessary for the welfare of our people.

The Association's Council on Research, although not so old, has been active in stimulating continuing education on animal research throughout the nation.

Several years ago the Council on Research recommended that the American Veterinary Medical Association establish a research trust fund which would provide financial aid to selective veterinarians to help them secure additional
training needed for a career in research or teaching or both in any discipline within the broad field of veterinary medicine. This research trust fund was established and has been supported from donations by members of the Association and others interested in the program.

Last Spring the Council on Research received 23 applications for fellowship grants from qualified veterinarians to enable them to continue their training and research. The Research Council was barely able to allocate sufficient funds to support 11. These requests for fellowships at an expenditure of 35 thousand, and of the 11, four were for renewal grants. It is unfortunate, the size of the individual stipend is below the average of most stipends granted to fellows of equal training.

A point of major concern in this important activity was that a comparable program for the 1961-62 year will deplete the available funds. To meet this existing emergency, I should like to point out that at the Ninety-Seventh Annual Convention in Denver, the Women’s Auxiliary of American Veterinary Medical Association adopted a program of assistance wherein they will launch a nation-wide campaign soliciting 75 thousand in funds to aid research. This is an ambitious project and should merit your support.

I am sure it is unnecessary for me to go into the advantages of additional training for the profession, or an increased animal diseases research. To assist with this program, the Council on Research has prepared a new brochure entitled “New Frontiers in Veterinary Medicine” and has made studies of undeveloped areas of research in veterinary medicine, ways and means to stimulate an interest in research, and a review of veterinary research that is being now pursued. This brochure has been sent to members of the profession and we shall hear more of the research studies during this year.

Several years ago, the American Veterinary Medical Association launched a public relations program through which it was hoped that all people would be informed of veterinary science and its services available to them. The movement has caught on, through State Association activities and considerable effort is being made to properly inform the public about veterinary medicine through television and radio programs, the press, and other means. To facilitate and coordinate the public relations effort, the American Veterinary Medical Association set up a professional relations department in the headquarters office to meet with allied professions and organized groups related to agriculture and animal health. The American Veterinary Medical Association is, therefore, constantly working with national and professional groups, trade associations, farm organizations, and state medical societies on matters of mutual interest. It cooperates with Federal agencies such as the United States Department of Agriculture, United States Department of Health and Welfare, the Armed Forces, the Food and Drug Administration, the Selective Services, and the Federal Defense Administration. These activities serve to promote a better understanding of the veterinary profession and its functions as it relates to the welfare of the people it serves.

While considerable progress has been made in the public relations, it is the ambition of the Association to further this program during the coming months.
Since I have been a member of the United States Livestock Sanitary Association for many years, I am quite aware of the many activities and problems of the Association, of the good work the Association has done for the livestock industry and the general public in its areas of continued education, animal diseases control and eradication, and development of uniform regulation for the movement of livestock. The over all program has been a tremendous one and we all realize that considerable progress has been made in meeting goals that were set years ago, yet much remains to be done.

Realizing that there remains much to be done for the livestock and poultry industries, the general public, the veterinary profession, I should like to leave with you some specific suggestions to think about, that in my estimation, are deserving of your continued study and action.

First. It seems to me that a program of continued education designed specifically for veterinarians engaged in regulatory veterinary medicine, including the veterinary practitioner would be appropriate. I am well aware of the shortcomings of the veterinary schools in the instruction of regulatory veterinary medicine, but I hasten to say the schools are now doing a better job than ever before. This is not enough, however, because it is impossible for any veterinary school to set up sufficiently strong programs in the many areas of instruction to turn out specialists in each area and in addition, regulatory veterinary medicine is constantly changing, which makes a program of continued education necessary.

I do not propose how this should be done except to suggest the possible consideration of implementing graduate curricula, short courses, conferences, and special meetings to be designed primarily for those veterinarians engaged in regulatory medicine, including practitioners concerned with the regulations.

I would suggest also that this continued educational program include a session on public relations. In the past, regulatory veterinarians have been accused of arrogance and having a big stick attitude. This, of course, was unfortunate, was not conducive to good practices, and undoubtedly impeded programs in certain states. The public is entitled to, and we are obligated to provide a competent, courteous veterinary service.

Second. I would urge that this Association, in cooperation with appropriate Federal and state agencies, continue to strive for improved and simplified uniform regulations concerned with the movement of livestock, poultry and pets, on a national and an international basis, and that these same groups also continue their effort toward providing a more simplified, yet comprehensive and complete health certificate. This program should not usurp state rights nor should it diminish the individual state responsibilities concerned with the movement of livestock, poultry, and pets.

Third. The United States Department of Agriculture estimates that the economic losses to this nation in animal diseases and parasites is over one billion dollars per year. Insect pests of livestock and poultry are believed to account for more than five hundred million dollars of this loss. When we think of the great progress made during the past several years in the development of parasiticides, this economic loss is shameful and disgraceful.
I propose, therefore, that the United States Livestock Sanitary Association give serious consideration to taking the leadership to initiate and stimulate a program of total eradication of external animal parasites. I am conscious of the many and great problems that would arise from such a program, its immediate cost, the cooperative effort that would be necessary for the implementation, and of the education and publicity that would be required in order that the livestock industry accept it.

It seems to me, however, that such a program should be started and that perhaps the most logical starting place would be for all states to provide legislation requiring that all animals entering the state, except for slaughter, be free of external parasites. This, of course, would call for an extensive dipping program of animals for interstate shipment. Once the first phase of the program got under way, it should then be followed by an intrastate external parasite eradication program.

This second phase, of course, would be a tremendous program within the state. There is no question in my mind, however, but what it is just as feasible to eradicate external parasites as it is to eradicate Brucellosis and other diseases.

Fourth. I would recommend that the United States Livestock Sanitary Association spearhead a cooperative movement on the part of state and Federal regulatory agents in striving for perfection of intrastate regulatory services to the extent that such services become entirely relied upon. If such perfection were forthcoming, then there would be no need for one state to place an embargo upon another state for the movement of livestock because of the localized outbreak of sheep scab, or cattle scab, or some other disease.

State and local regulatory agencies, therefore, could be depended upon to circumvent the effective area of quarantine areas and thus allow the interstate movement of livestock from the uninfected areas of the unfortunate state. Such practices are being followed in some instances, but nevertheless the situation could be greatly improved.

Fifth. We have often been informed that the general practitioner of veterinary medicine is the backbone of the veterinary profession. It is true that in animal diseases eradication and control programs, the general practitioner is an important cog. It is my belief, therefore, that the services of a general veterinary practitioner could be utilized more efficiently and to a greater extent than in the past and that this utilization could be placed on a more congenial, cooperative, and satisfactory basis.

United States Livestock Sanitary Association, having a large membership of regulatory veterinarians, could, therefore, stimulate a program of better and more efficient cooperative relationship with the general practitioner.

In enumerating the points above, I realize that the United States Livestock Sanitary Association can not, in this, implement any of the recommendation, but it does have the potential of influence for recommending, stimulating, and leading the way for each program.

I thank you very much.
MEMBERS of the United States Livestock Sanitary Association, Distinguished Guests, Ladies and Gentlemen: The 64th Annual Meeting of this Association is now well under way, and it is my privilege and pleasure to welcome each of you on behalf of this Association. Our history tells us that this is our first trip to West Virginia and I want to express my thanks to Commissioner Johnson and particularly Tom Green for the arrangements and plans which I know will make this meeting successful. Many of us have thought of this great state as being synonymous with coal, but in recent years foresight by agriculturists have started to utilize abandoned mines for added agricultural income to the people of West Virginia. I refer principally to mushroom and broiler production.

When I served as the Chief, Regulatory Official and Director of the Department of Agriculture in Ohio, I always found the same officials in West Virginia most cooperative and willing to sit down around the table and work out the many mutual problems common to states which are neighbors.

To those of you who are attending this meeting for the first time as an official from your state or as a visitor, I urge you to participate in the deliberations of this Association, particularly in the Committee activities which might be dealing with a subject of particular interest to you. The United States Livestock Sanitary Association deals primarily with disease control and disease eradication and many of the Committee deliberations lead to ultimate national policy in the control and eradication of these diseases.

I have made a review of the Presidential addresses of my predecessors over the past 12 or 15 years and by so doing cannot fail to recognize the progress which has been made by this Association. We have changed the format over the years from what was strictly a research workers' conference to an Association which delves into many areas, but with constant emphasis on protecting the great livestock and poultry industries from disease and to advance these industries in their service, not only to this nation, but to many parts of the world.

Today, I want to change the format of my predecessors just a little. I will not attempt to elaborate on the past, present or hopes for the future by dealing with the specific disease problems which are constantly before the regulatory officials and livestock industries. I prefer to devote what time I have to talking with you, our members, about this Association and its present and future.

To look at the United States Livestock Sanitary Association and its affairs objectively, you are faced with a situation which appears to be a paradox.
First you meet the critic who tells you the Association affairs are placed too much in the hands of the regulatory officials and that the livestock producer doesn’t have an opportunity to express his views. Secondly, you will meet the member who believes in the Association and derives satisfaction from his contributions over the years. Objectively, both opinions are justified. By constitutional amendment, the governing body of this Association has been enlarged to provide seats on the Executive Committee for eight representatives of the various livestock and poultry industries. This was accomplished last year in San Francisco and provides two representatives each from the four regions into which this Association is divided. It is the hope of those who brought about this change, that producer members of the United States Livestock Sanitary Association will take a greater interest in the many problems brought to the floor of this convention by learning who their representatives on the Executive Committee are, and either by voicing his opinions or problems in the various committee hearings, or through these representatives let his wishes be known.

At this point, I would like to thank Doctor Hendershott for formulating an excellent program. A program which in my opinion is pretty tight time-wise but one which covers many areas of interest. Along this line, let us ask ourselves this question. We constantly urge livestock producers to participate in this meeting, but does the program which the United States Livestock Sanitary Association present each year truly appeal to the livestock producer? Does it offer him the incentive to travel 100 or 1,000 miles or more at his own expense to participate in our deliberations?

I would therefore recommend a modification of our future annual meeting program to develop a program which will have more appeal to the nation’s farmer and rancher. However, by so doing it places a greater responsibility on all members to see that agricultural leaders in their respective states are aware of the meeting, where it is to be held, what subject material on the program is of particular interest to them and finally to urge their attendance. I would also urge greater participation by the officers of this Association, working with the Committee Chairman, as provided by our constitution in the formulation of future annual programs.

These thoughts quickly lead me to matters of membership. During recent years the membership of this Association has been averaging between 1,000 to 1,100 members per year. Why? Is the answer in the representation critique, the type of program offered or a lack of effort on the part of the present membership to interest new members? Certainly our Secretary has attempted to stimulate each state regulatory official when the annual meeting was in their particular area to develop interest. Perhaps this is not enough. Therefore, I recommend to the Executive Committee and officers of this Association that a specific committee on Means and Goals for membership be activated for a minimum period of five years to work out methods to stimulate new membership for this Association. If such a project is not considered immediately, the future of the United States Livestock Sanitary Association could be in doubt.
Next, a comment or two regarding the Committees of the Association. Some of you may recall that at San Francisco I named committee chairmen. The idea behind this plan was to give the chairmen an opportunity to pick their committee members from qualified people in the field in which their committee would operate. For the most part, this worked quite well, and I want to express my appreciation to the Chairmen and members of all committees who accepted the request to serve during the last year. Some people facetiously say, "to bury a project, appoint a committee." This philosophy does not apply to this Association. Our progress stems from the long hours and arduous effort, particularly immediately preceding and during the annual meeting. For several years, I have tried to determine how the number of committees might be reduced. It seems however, that for every one whose services are terminated at least two new committees spring up. I would recommend to the officers who will follow in the years to come that they scrutinize the Association's needs carefully before appointing new committees, as many times new projects fit nicely into old long established working committees.

Vigorous leadership and continuing, ever increasing acceptance of responsibility by all members in areas of increased membership, committee assignments and programing for annual meetings are essential to the growth of the Association.

Finances of the Association deserve and require mention at this time. Last year the Executive Committee issued a mandate for the officers to dispose of the shares of Columbia Gas common stock, which had been purchased by selling shares of American Telephone and Telegraph. This was accomplished in mid-August of this year when the Columbia Gas stock reached the price paid for it the year before. Also the Executive Committee requested that these funds be placed in insured accounts. Because of the different types of accounts available, your officers felt that further instruction should be obtained from the Executive Committee before a reinvestment program for the Association is established. It is my recommendation that 4/5ths of the assets be placed in United States Government Bonds and the remainder be placed in an insured savings account to be selected by the Secretary-Treasurer. Both the bonds and accounts to be established in the name of the United States Livestock Sanitary Association and over the signature of all the officers of this Association for the coming year. The finances of the Association seem to be at a level which are insufficient for us to progress rapidly into new areas but are adequate to maintain our present needs. Added membership should improve this situation. Our growth over the past few years indicates that we should operate on a budget approved annually by the Executive Committee, therefore, I recommend that the Secretary-Treasurer submit at the first meeting of the Executive Committee at each annual meeting a budget covering the operation of his office for the coming year, travel expenses of the elected officers on the Committee on Program and Policy when meeting in Washington, travel expenses of the President when representing this Association, particularly at the National Association of State...
Departments of Agriculture, printing costs of the annual proceedings, and any other items which contribute to the benefit and progress of this Association.

As I stated in the beginning, I purposely have not mentioned programs or problems dealing with specific disease problems. I did so knowing that as each committee report is given during the next few days that these subjects will be adequately covered. However, I would be remiss if mention was not made of the progress which has been made during the last six years in the relationship between this Association and the United States Department of Agriculture. You may remember that last year the Committee on Legislation and the Agricultural Research Service Advisory Committee were combined into the Committee on Program and Policy. This group is made up of the officers and representation from that regional group which is not represented by an officer. The President is not a member and the first vice-president serves as chairman. The opportunity for this group to meet with officials of Agricultural Research Service on budget, programs and legislation gives a better understanding and lends support to programs at a national level.

It has been a distinct privilege for me to be afforded the opportunity to serve as your President during this past year. The cooperation of the other officers, the committees and membership is most appreciated. I again want to thank Tom Green and his staff and Ralph Hendershott for their efforts in planning this meeting. I know we all will leave at the end of the meeting better prepared to cope with the problems we all face in protecting the interests of our great livestock and poultry industries. Thank you.
PRESENTATION OF KEY TO PRESIDENT HAY

R. A. HENDERSHOTT, Secretary-Treasurer

As has been customary during the past decade those men who have preceded you as President of this Association have each been the recipient of a key or tie pin made from the Association die as a memento of their service.

A review of the long list of the outstanding men who have served with distinction as President are listed in the front of the Annual Proceedings and should serve as an inspiration to any person who aspires to leadership in this Association.

It is my privilege to present to you this tie holder, emblematic of your service as President in 1960.

Dr. J. R. Hay: Thank you, Doctor Hendershott. I hope to measure up to my predecessors in the conduct of this 64th Annual Meeting.
Mr. President, Members and Guests of the United States Livestock Sanitary Association: This past year has been a memorable one for many reasons.

First, the attendance at the San Francisco Meeting exceeded our expectations in that 304 persons registered and attended both the meeting of the Diagnosticians and the three-day sessions of the Sixty-third Meeting of this Association.

Second, we shall remember the San Francisco Meeting for the amendment to our Constitution and By-Laws which made possible the addition of two farm representatives from each of the four districts of the United States to our Executive Committee. At this meeting we take pleasure in welcoming the following farmer representatives who were elected to serve their respective districts. The northeast will be represented by Mr. F. J. Nutter of Corinna, Maine, a dairyman and former Secretary of Agriculture of Maine, and by Mr. Hubbard, a prominent poultryman of the northeast from Walpole, New Hampshire. The southern states will be represented by Mr. J. Armstrong, a prominent livestock producer from Selma, Alabama, and member of the American National Livestock Association, and by Mr. J. B. Nance, a swine producer from Alamo, Tennessee, and President of the National Swine Growers Council.

The north central states will be represented by Mr. M. Steddom of Iowa, a swine breeder, and by Mr. A. W. Agnew, of Milton Junction, Wisconsin, a dairyman of prominence in that area.

The western states will be represented by two well known livestock men, members of long standing in our Association, namely, Mr. J. S. Brenner of Grant, Montana, a cattle breeder and State Legislator currently campaigning for re-election, and by Mr. O. H. Timm of Dixon, California, a prominent sheep breeder in that area.

We all welcome these men on our Executive Committee and their assistance as well as members of the various disease committees to which they have been appointed.

I shall remember the San Francisco Meeting especially for the reporter who walked out when we needed her most. It occurred at the beginning of the Scrapie panel for which none of the participants had formal written papers. Fortunately, we were experimenting with tape recording the meeting and we had the tapes of the meeting. Transcription from the tape was time-consuming as the remarks had to be handwritten, then typed and edited.

Our hog cholera program got under way in March as a result of a discussion which took place at the February Meeting of L. C. I. in Chicago. At this meeting I suggested that the Extension Service and the Agricultural Research Service of the United States Department of Agriculture
jointly arrange to conduct regional meetings on hog cholera similar to and patterned after those held to promote the eradication of brucellosis.

The initial meeting was held in Chicago in March and a second one in New York and the third one in New Orleans. I attended the first two and was disappointed in both for the reason that too few swine producers were present at either.

We do not need meetings to discuss hog cholera eradication with regulatory officials. We do need a number of meetings at state and county level for swine producers, feeders and breeders, so that they may learn the facts and be encouraged to enthusiastically cooperate in a hog cholera eradication program.

It is long past the time that our farmers should stop paying tribute to a disease that can and should be eradicated. There is sufficient information at hand to accomplish eradication and our Committee on the Nationwide Eradication of Hog Cholera has set forth the facts; we need only to implement them.

On Sunday and Monday, October 15 and 16, we were privileged to attend an excellent program on the laboratory diagnosis of Tuberculosis. Those that availed themselves of the opportunity to be present at this very impressive and instructive meeting were well repaid.

It is unfortunate that we cannot afford to publish the proceedings of the tuberculosis meeting. I understand that the United States Department of Agriculture, sponsors of the meeting, will publish an abstract of it.

The Fourth Annual Meeting of the Conference of Veterinary Laboratory Diagnosticians was held here on Monday and Tuesday, and the Laboratory Diagnosis of Tuberculosis was a part of their Conference. On the last page of your program you will find a list of the papers presented here yesterday.

You will note from our financial report that we operated at a deficit this past year. Perhaps we should increase the registration fee, few association meetings charge less than a ten-dollar fee.

There is also a recommendation by our Accountant that our Constitution and By-Laws be amended as follows:

Line 89. That with the exception of a change in the name of this Association, upon the dissolution of this Corporation or the termination of activities thereof all remaining net assets thereof shall be contributed for the utilization in the advancement and research of infectious diseases of animals and no part of the net assets shall inure to any person or group of persons for private gain.

I wish at this time to express my appreciation to the officers and members for their cooperation and, more particularly to the Chairmen and members of the various committees who served us so well during 1960. My thanks also to Drs. T. C. Green and A. S. Barnes and the young ladies from their offices for their assistance at this Sixty-fourth Annual Meeting.

As usual it has been a pleasure for me to serve the Association as Secretary-Treasurer the past year.

Following is a report of our auditor:
REPORT OF THE AUDITOR

C. BERGEN GROENDYKE
CERTIFIED PUBLIC ACCOUNTANT

Trenton 8, New Jersey

Dr. Ralph Hendershott
Secretary-Treasurer
United States Livestock Sanitary Association
Trenton, New Jersey

October 11, 1960

Dear Sir:

From the books and records of the United States Livestock Sanitary Association, I have prepared a statement of cash receipts and disbursements for the period from December 8, 1959 to October 10, 1960.

The cash balance at the end of the period was reconciled to the actual bank balance in the First Trenton National Bank as shown on the bank statement as of September 30, 1960. Separate sheets show a statement of Net Worth and a summarized Statement of Operations prepared from figures found in your cash books, plus other information given to me by you.

I have not had sufficient time to adequately review the status of this corporation in its relationship to the Internal Revenue Code and Regulations. The corporation’s “Certificate of Incorporation” states that “This corporation shall not be for profit nor shall it have any capital stock.” If it has not already been done, I would suggest that a resolution be passed by the Board of Directors and/or the Executive Committee to the following effect:

“That upon the dissolution of this corporation, or the termination of activities thereof, all remaining net assets thereof be contributed for utilization in the advancement and research of infectious diseases of animals.”

This added provision further exemplifies the complete non-profit status of your organization in assuring that the benefits of this corporation will never inure to any individual person or group of persons. It will be added strength if such a statement could be made a part of the by-laws through an amendment thereto.

Respectfully submitted,

C. BERGEN GROENDYKE,
Certified Public Accountant.
## UNITED STATES LIVESTOCK SANITARY ASSOCIATION

### STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE PERIOD
### FROM DECEMBER 8, 1959 TO OCTOBER 10, 1960

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balance in First Trenton National Bank, December 8, 1959</td>
<td>$263.47</td>
</tr>
<tr>
<td>Increaser by Cash Receipts:</td>
<td></td>
</tr>
<tr>
<td>Individual Dues</td>
<td>$5,396.95</td>
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<tr>
<td>Official Dues</td>
<td>2,700.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>1,788.70</td>
</tr>
<tr>
<td>Circular 1</td>
<td>3.45</td>
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<tr>
<td>Foreign Annual Disease Handbook</td>
<td>394.74</td>
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<tr>
<td>Hog Cholera Pamphlet (&quot;What One Should Know About Hog Cholera&quot;)</td>
<td>2,896.86</td>
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<tr>
<td>Reprints</td>
<td>1,064.57</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>1,540.00</td>
</tr>
<tr>
<td>Dividend</td>
<td>1,050.00</td>
</tr>
<tr>
<td>Brucellosis Facts (&quot;What is Known About Brucellosis&quot;)</td>
<td>6.00</td>
</tr>
<tr>
<td>Total Cash Income</td>
<td>$16,841.27</td>
</tr>
<tr>
<td>Transfer Funds from United Savings and Loan</td>
<td>3,937.49</td>
</tr>
<tr>
<td>Total Cash Receipts</td>
<td>$20,778.76</td>
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<tr>
<td>Decreased by Cash Expenditures:</td>
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<tr>
<td>Meeting Expense</td>
<td>$1,150.24</td>
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<tr>
<td>Printing and Stationery</td>
<td>8,159.18</td>
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<tr>
<td>Salary and Bonus</td>
<td>7,500.00</td>
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<tr>
<td>Travel</td>
<td>2,465.34</td>
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<tr>
<td>Communications</td>
<td>384.44</td>
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<tr>
<td>Miscellaneous</td>
<td>496.09</td>
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<tr>
<td>Insurance</td>
<td>136.44</td>
</tr>
<tr>
<td>Returned Check</td>
<td>5.00</td>
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<tr>
<td>Total Expenditures for 1959-1960</td>
<td>$20,536.73</td>
</tr>
<tr>
<td>Prior Year's Printing Invoices</td>
<td>1,256.09</td>
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<tr>
<td>Total Cash Expenditures</td>
<td>21,792.82</td>
</tr>
<tr>
<td>Balance in First Trenton National Bank, October 10, 1960 (Deficit)</td>
<td>($750.59)</td>
</tr>
</tbody>
</table>
REPORT OF

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS FOR THE PERIOD FROM
DECEMBER 8, 1959 TO OCTOBER 10, 1960

Income:
Cash $16,841.27
Receivables 800.25

$17,641.52

Expenditures:
Cash $20,536.73
Payables (Printing) 1,093.28

21,630.01

Deficit from Operations for Fiscal Period ($3,988.49)

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

NET WORTH — OCTOBER 10, 1960

Balance in First Trenton National Bank (Deficit) ($750.59)
Check, Columbia Gas Company 27,900.58
United Savings and Loan, Deposit 1.00
Furniture and Fixtures 640.00
Accounts Receivable 800.25

$28,591.24

Less: Accounts Payable (Printing) 1,093.28

Net Worth, October 10, 1960 $27,497.96
MEMORIAL SERVICE
M. N. Riemenschneider
Oklahoma City, Oklahoma

Mr. President, Members of the Association, Distinguished Guests, Ladies and Gentlemen: We now come to that part of the program in which we pause to pay our respects to our departed colleagues who can be with us in spirit only on this day.

We have sustained real losses this past year by the passing of the following men who have contributed a great deal to this organization:

WAYNE A. ANDERSON

Dr. Wayne A. Anderson (CSU '36), Denver, Colorado, passed away, May 15, 1960, after an extended illness.

Doctor Anderson accepted a position with the former Bureau of Animal Industry in 1936. He served with the exception of one (1) year, for various periods in Indiana, Missouri, North Carolina and Washington, D. C., on brucellosis and tuberculosis work, and in Mexico on foot and mouth disease eradication. In 1947 he transferred to the Bureau of Animal Industry Laboratory at Denver, which later became the Animal Disease and Parasite Research Laboratory where he did research in pathology of diseases of livestock. He served as Director of the laboratory from July 1956 to August 1958 when he was permitted to relinquish the duties because of ill health.

Doctor Anderson was author or co-author of many contributions to veterinary literature. He was affiliated with many professional organizations one of which was the American College of Veterinary Pathologists.

TRUMAN W. COLE

Dr. Truman W. Cole (CVC '15), 65, Jacksonville, Florida, passed away on January 19, 1960. At the time of his death, he was veterinarian in charge of the Florida field station for animal disease eradication and animal inspection and quarantine in Jacksonville.

Born in McKinney, Texas, Dr. Cole was appointed a veterinary inspector for the United States Department of Agriculture, the same year he graduated from veterinary school. In 1927, he was named veterinarian in charge of the Florida field station at Jacksonville. Ten years later, he became assistant chief of the field inspection division, Bureau of Animal Industry, in Washington, D. C. He was inspector in charge, port of New York and superintendent of the Animal Quarantine Division in Clifton, N. J., from 1944 to 1949.

Dr. Cole returned to Washington in 1949 when he was made chief of the interstate inspection division. In 1955 he became chief of the public stockyards section and an area director for the Animal Disease Eradication Di-
vision, a position he held until 1958 when he was placed in charge of the Florida field station.

In 1956 the Department presented Dr. Cole with an award for outstanding service in recognition of his contributions to the field of animal health. He was the author of a number of Department publications.

JAMES G. FISH, SR.

Dr. James G. Fish, Sr. (OSU '21), 59, Jacksonville, Fla., died as the result of a heart attack while visiting his farm in Quitman on March 1, 1960.

A native of Chicago, Dr. Fish moved to Jacksonville following his graduation from Ohio State in 1921. He had been associated with the city board of health for 29 years. Dr. Fish was instrumental in developing a program to eradicate tuberculosis in dairy herds and later became chief inspector for the health department as well as operating his general veterinary practice. For the last few years he had been in partnership with one of his five sons, Dr. James G. Fish, Jr. (TEX '54).

ARTHUR D. GOLDAFT

Dr. Arthur D. Goldhaft (UP '10), 74, Vineland, N. J., died April 2, 1960, following a long illness.

In 1914 Dr. Goldhaft founded the Vineland Poultry Laboratory wherein he devoted himself to poultry disease prevention. In 1937 he helped to develop vaccines against Asian influenza. In his work, he cooperated with the late Dr. Frederick R. Beaudette of Rutgers University.

Dr. Goldhaft worked to promote the agricultural economy of Israel and had held many responsible positions in the Zionist Organizations of America. In 1951 he was the United States representative to the World Poultry Congress in Paris and, in 1957, the Vineland community awarded him a plaque for his assistance to farmers. He is a past president of the Animal Health Institute of Chicago.

IVAN G. HOWE

Dr. Ivan G. Howe (COR '14), Belmont, New York, passed away at the Veterans Administration Hospital in Buffalo on January 29, 1960. He was 67 years of age.

Dr. Howe retired as Director of the New York State Division of Animal Industry in 1954, after serving in that capacity since 1943.

He held the post of Welfare Commissioner of Allegany County for several years before assuming the position as Director of the New York Division of Animal Industry.

He was 1st Vice-President of the United States Livestock Sanitary Association in 1953-54 and served on several of its committees.

He was a member of a number of professional organizations.
MEMORIAL SERVICE

GUSTAVE LABELLE

Dr. Gustave Labelle (MON ’18), 62, St. Hyacinthe, Que., Canada, Director of the School of Veterinary Medicine at the University of Montreal, died in an automobile accident at Fabreville on February 5, 1960.

Born in St. Eustache, Dr. Labelle began his studies at the College of St. Eustache. In 1915 he entered the School of Veterinary Medicine, which at that time was in Montreal, and in 1918 at the age of 21, obtained his degree in veterinary medicine.

In September, 1918, Dr. Labelle opened his practice in Saint Eustache, succeeding his father. In 1928 the School of Veterinary Medicine, then located at Oka, asked him to join their teaching staff, and in 1942, he was nominated director of studies there. In 1947, when the school was established at Saint Hyacinthe, Dr. Labelle became director of the School, the position he held at the time of his death.

An active man, devoted to the public cause and to his profession, he was connected with many councils and organizations. He was mayor of St. Eustache, president of the school commission, a member of the study commission of the University of Montreal, a member of the Board of Governors of the College of Veterinary Medicine in Quebec and a former president and co-founder of the Society of Veterinary Medicine of the Province of Quebec.

Dr. Labelle was the author of several professional books.

WILLIAM C. LOGAN

Dr. William C. Logan, 68, Urbana, Illinois, passed away on June 22, 1960, as a result of a heart attack. At the time of death Dr. Logan was a Veterinary Livestock Inspector with the Animal Disease Eradication Division of Agricultural Research Service, assigned to TB eradication in problem herds over the State of Illinois, which position he had held since 1954.

Dr. Logan was graduated from St. Joseph Veterinary College, St. Joseph, Missouri, in 1922, following preliminary studies at Peru State Teachers College, Peru, Nebraska, and at the University of Nebraska in Lincoln. He practiced veterinary medicine in Nebraska from 1922 to November, 1934, when he became a meat inspector with the Meat Inspection Division, Kansas City, Missouri. In February 1935 he began his work with the Animal Disease Eradication Division as a junior veterinarian in the TB Control program in Nebraska. He then worked on that program in Puerto Rico from June, 1936, to June, 1939. Dr. Logan came to Illinois in June 1939 and worked on Avian TB surveys until July, 1950, when he took over his last assignment, specializing in the cervical tuberculin test in problem herds with special attention to Johnne’s Disease. He was well known throughout the State as a specialist in this field.

EDWARD RECORDS

Dr. Edward Records (UP ’09), 73, died of a heart attack in his laboratory office on the University of Nevada campus, May 13, 1960.
MEMORIAL SERVICE

Dr. Records was executive officer of the Nevada State Department of Agriculture and director of the Animal Disease Laboratory. Prior to this appointment, he had been head of the Department of Veterinary Science at the University.

In 1914, Dr. Records was affiliated with the H. K. Mulford Co., Glenolden, Pa., as a bacteriologist when he was asked to come to Nevada to study "red water," a disease which was threatening the cattle industry in Carson Valley.

What was to have been a six-month project turned into seven years of research and resulted in discovery of the cause and nature of the disease as well as the development of a successful method of immunization which is still in use. Dr. Lyman Vawter was his associate in this work.

Research into many other livestock diseases and the contribution of over 30 publications to the literature of veterinary science helped to fill the 46 years of his service to the State of Nevada. He was an executive officer of the Nevada State Board of Stock Commissioners for many years. He contributed greatly to the animal disease control programs of the state, especially, in later years, in the promotion of the state's campaign for brucellosis eradication.

He served as president of this Association in 1935.

The Twelfth International Veterinary Congress Prize, the highest honor in the field of veterinary medicine, was awarded to Doctor Records by the American Veterinary Medical Association in August, 1957.

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

SILENT PRAYER

Thank you, Ladies and Gentlemen, for your respectful participation.

All of these men served well the Livestock Industry of this great Nation, unselfishly contributing of their time, efforts and energies in their respective fields.

Let us be thankful for the opportunity and privilege of having known them; of working with them; for the contribution they have made in their respective fields of endeavor and the ideals they have set forth.

Even though we are deprived of the privilege of personal contact, let us cherish their memory and the ideas they have passed on to us and let us not falter in ever striving to better serve to the very best of our ability in reaching higher plateaus of accomplishments.

Thus let us memorialize our departed colleagues this day.
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS


For a good many years this Committee has attempted, in many ways, to obtain more uniformity in the livestock import regulations of the various states. Last year the Committee suggested that an approach be made on a regional basis. Your Committee, following the San Francisco meeting, wrote all state livestock disease control officials suggesting that an effort be made at all regional meetings to obtain a greater degree of uniformity within their respective regions.

The regional approach, in the western states, met with considerable success in obtaining uniformity particularly in regard to cattle brucellosis requirements. The southern states devoted considerable time on this subject at their New Orleans meetings. The results of their deliberations have been mailed to all livestock sanitary officials in the form of two resolutions.

As a result of these resolutions and other requests the United States Department of Agriculture, Agricultural Research Service, has proposed an amendment to Title 9 which is appended to this report.

This Committee recommended that those portions of Title 9 that permit the interstate movement of livestock on owner's or shipper's permits be eliminated.

Your Committee wishes to urge continued efforts be given by regional groups to study and implement more uniformity of import livestock health requirements between the states in their respective regions. Once the states, within geographical regions, can reach more uniformity, then the task of this Association to obtain more uniformity between the geographical regions, will not be as insurmountable as attempting to obtain uniformity between fifty individual states.

**TITLE 9 — ANIMALS AND ANIMAL PRODUCTS**

**CHAPTER I — AGRICULTURAL RESEARCH SERVICE, DEPARTMENT OF AGRICULTURE**

**SUBCHAPTER B — COOPERATIVE CONTROL AND ERADICATION OF ANIMAL DISEASES**

**SUBCHAPTER C — INTERSTATE TRANSPORTATION OF ANIMALS AND POULTRY**

**PART 51 — CATTLE DESTROYED BECAUSE OF BRUCELLOSIS (BANG'S DISEASE), TUBERCULOSIS, OR PARA-TUBERCULOSIS**
REPORT OF COMMITTEE

PART 78 — BRUCELLOSIS IN DOMESTIC ANIMALS

AMENDMENTS TO REGULATIONS GOVERNING THE INTERSTATE MOVEMENT OF
DOMESTIC ANIMALS BECAUSE OF BRUCELLOSIS, AND PAYMENT OF
INDEMNIETY FOR CATTLE DESTROYED BECAUSE OF BRUCELLOSIS,
TUBERCULOSIS, OR PARA-TUBERCULOSIS

Pursuant to the provisions of sections 4, 5, and 13 of the Act of May 29, 1884, as amended, sections 1 and 2 of the Act of February 2, 1903, as amended, and section 3 of the Act of March 3, 1905, as amended (21 U.S.C. 111-113, 114a-1, 120, 121, 125), Part 51 of Subchapter B and Part 78 of Subchapter C, Chapter I, Title 9, Code of Federal Regulations, are hereby amended in the following respects:

1. Paragraph (m) of § 51.1 is amended to read:

(m) Official vaccinate. A bovine animal vaccinated against brucellosis while from four through eight months of age, on or before June 30, 1957, or a bovine animal of a beef breed in a range or semi-range area vaccinated against brucellosis while from four to 12 months of age, on or before June 30, 1957, under the supervision of a Federal or state veterinary official, with a vaccine approved by the Division; or a bovine animal vaccinated against brucellosis while from four through eight months of age, subcutaneously, on or after July 1, 1957, or a bovine animal of a beef breed in a range or semi-range area vaccinated against brucellosis while from four to 12 months of age, subcutaneously, on or after July 1, 1957, under the supervision of a Federal or state veterinary official, with five cc. of a vaccine approved by the Division; permanently identified as an “official vaccinate” for purposes of interstate movement by an official metal eartag affixed to the left ear of the animal, or by a tattoo, approved by the Division bearing the inscription “US”, together with serial numbers for each animal and letters which shall include code numbers or letters identifying the accredited veterinarian applying the eartag or tattoo, and reported at the time of vaccination to the appropriate State or Federal agency cooperating in the eradication of brucellosis.*

2. Paragraph (j) of § 78.1 is amended to read:

(j) Official vaccinate. A bovine animal vaccinated against brucellosis while from four through eight months of age, on or before June 30, 1957, or a bovine animal of a beef breed in a range or semirange area vaccinated against brucellosis while from four to 12 months of age, on or before June 30, 1957, under the supervision of a Federal or State veterinary official, with a vaccine approved by the Division; or a bovine animal vaccinated against brucellosis while from four through eight months of age, subcu-

* The eartags and an instrument for applying the tattoo shall be furnished the accredited veterinarian but shall remain the property of the Division and be withdrawn from his possession when and if the veterinarian’s accreditation is withdrawn. Appropriate records as to each eartag or tattoo applied shall be maintained by the accredited veterinarian applying such eartag or tattoo.
taneously, on or after July 1, 1957, or a bovine animal of a beef breed in a range or semi-range area vaccinated against brucellosis while from four to 12 months of age, subcutaneously, on or after July 1, 1957, under the supervision of a Federal or state veterinary official, with five cc. of a vaccine approved by the Division; permanently identified as an "official vaccinate" for purposes of interstate movement by an official metal eartag affixed to the left ear of the animal, or by a tattoo, approved by the Division bearing the inscription "US", together with serial numbers for each animal and letters which shall include code numbers or letters identifying the accredited veterinarian applying the eartag or tattoo, and reported at the time of vaccination to the appropriate state or Federal agency cooperating in the eradication of brucellosis.

3. Paragraph (c) of section 78.3 is redesignated as paragraph (d) and a new paragraph (c) is added to read:

§ 78.3 (c) Whenever the regulations in Parts 71, 72, 73, 74, 76, 77, 78, 79, 80, 81, 82, and 83 (9 CFR) require a certificate in connection with the interstate movement of animals, there shall be issued by an accredited veterinarian or Federal or state inspector, a Universal Health Certificate acceptable to all states and territories; such certificate shall show the identification code number of the accredited veterinarian issuing the certificate, the name and address of the consignor and consignee, the identification tag number, tattoo, or registration number of each animal or other proper identification, and the specific class in which the animals fall.

Any person who wishes to submit written data, views, or arguments concerning the foregoing proposed regulations may do so by filing them with the Director, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Washington 25, D. C., within 30 days after publication hereof in the Federal Register.

Done at Washington, D. C., this day of October, 1960.

Administrator
Agricultural Research Service
REPORT OF THE COMMITTEE ON NOMINATIONS


Your Committee on Nominations presents the following slate of officers for 1961 for your consideration:
President: A. P. Schneider, Boise, Idaho
President-Elect: W. L. Bendix, Richmond, Virginia
First Vice-President: T. J. Grennan, Providence, Rhode Island
Second Vice-President: A. K. Merriman, Springfield, Illinois

Industry Representatives:
Southern Region—
Mr. John Armstrong, Selma, Alabama
Mr. James Nance, Alamo, Tennessee

North Central Region—
Mr. M. Steddom, Grainger, Iowa
Mr. A. W. Agnew, Milton Junction, Wisconsin

Western Region—
Mr. J. S. Brenner, Grant, Montana
Mr. O. H. Timm, Dixon, California

Eastern Region—
Mr. William G. Mennen, Chester, New Jersey
Mr. W. Alton Jones, Easton, Maryland

As is customary, the Chair recognizes nominations from the floor. Do I hear any?

DOCTOR GREEN: I move that the nominations be closed.

DOCTOR BODENWEISER: I second the motion.

PRESIDENT HAY: All those in favor, signify by the usual sign. All those opposed? The motion carries. The maker of the motion did not indicate that the Secretary cast the unanimous ballot for this slate of officers, so I will instruct him to do so at this time.

DOCTOR HENDERSHOTT: I hereby cast the unanimous vote of the Association for the officers and representatives for the areas as represented on the blackboard.

PRESIDENT HAY: Thank you, Doctor Hendershott.

Will you escort the officers forward?

Installation of officers.
PRESIDENT HAY: It is a pleasure to welcome this corps of officers. I certainly feel that the affairs of the Association will rest in good hands and I certainly hope the members of the Association will give them the same degree of cooperation they have given to me in the past year.

I believe it is customary that each one be given an opportunity to discuss his hopes for the future and his medical plans, so I will ask Timm as the representative from California if you care to say anything at this time.

MR. TIMM: This is certainly an unexpected opportunity. I thoroughly appreciate being nominated and selected as a representative. I hope that I, with other representatives will be able to contribute more in the future as we get to understand the philosophy and workings of the organization.

PRESIDENT HAY: Thank you.

Doctor Merriman being the newest member of the corps of officers, I want to congratulate you. I know that you must have a few words to say about this, if not about the great State of Illinois.

DOCTOR MERRIMAN: Thank you, President Hay, and members. I appreciate the honor bestowed on me and it will be my earnest desire to render this Association the best service that I can. Thank you.

PRESIDENT HAY: Tom, you have had a busy week. What do you care to say to the group at this time?

DOCTOR GRENNAI: Mr. President, members, I deeply appreciate this honor. For myself and for my state, I thank you very much.

PRESIDENT HAY: Doctor Bendix has the honor of being the first President-Elect of this Association. I think he can hold up pretty well to the responsibilities.

Bill, would you care to say a few words to the group?

DOCTOR BENDIX: Thank you, Jim.

I just want to say on behalf of the people—and I was corrected sharply recently for saying the State of Virginia, so I now say the Commonwealth of Virginia.

I thank you very much.

PRESIDENT HAY: Last but not least, your new President, Doctor Schneider. Duke, the responsibility is now yours.

DOCTOR SCHNEIDER: I am certainly most happy to have the honor bestowed on me and the confidence that you have shown in me. I know we look forward to a big year ahead of us. We have many obstacles to overcome and many things to solve.

I know with this grand slate of officers that I have behind me to help me, and with the splendid membership of all of us out here, we can look forward to a very good year. Thank you very much.

PRESIDENT SCHNEIDER: The Chair will entertain a motion to adjourn.
REPORT OF THE COMMITTEE ON FEDERAL PROGRAMS AND POLICY

DR. A. P. SCHNEIDER, Chairman, Boise, Idaho; DR. T. J. GRENNAN, Providence, Rhode Island; DR. H. G. GEYER, Columbus, Ohio; DR. W. L. BENDIX, Richmond, Virginia; DR. R. A. HENDERSHOTT, Trenton, New Jersey; DR. J. G. MILLIGAN, Montgomery, Alabama.

This Committee now known as the Committee on Federal Program and Policies continues to carry on all the functions of the former Committees known as the Advisory Committee of the United States Livestock Sanitary Association to the Agriculture Research Service, United States Department of Agriculture and the Legislative Committee of the United States Livestock Sanitary Association. The Committee will meet four times this year.

The first meeting was held February 24, 1960 in the Agriculture Building, Washington, D. C. The Committee listened, questioned and reviewed the remarks on the over-all research program of the Animal Disease and Parasite Research Division. Also, there was included an immediate situation report covering the preparedness program for diagnosing exotic diseases as related to the work at Plum Island. This report covered Foot and Mouth disease, Rinderpest, contagious Pleuropneumonia and some work on Teschen Disease. In the future work will be done on Sheep Pox, Rift Valley Fever and pneumonitis. No work at this time has been done on African Swine Fever.

The National Animal Disease Laboratory at Ames, Iowa, was discussed at length with regard to its present status in regard to building funds, equipment funds, staffing and getting into operation. Another item of outstanding importance was that the Committee was concerned with the need for considerable more research work being done in the area of chemical residues.

Animal Inspection and Quarantine activities were next discussed concerning the National Animal Disease Laboratory. Other problems reviewed included maintaining surveillance of foreign animal diseases and pests at ports of entry. This now also involves the St. Lawrence Seaway. The need of a West Coast Quarantine Station and additional facilities on the Canadian border were in turn discussed.

The Animal Disease Eradication Division activities were next discussed and this covered long range planning on the various eradication programs. The review basically covered all of their programs including Brucellosis, Tuberculosis, Screwworm, Scrapies, Anaplasmosis, VE and other miscellaneous diseases.

Following this the Meat Inspection Program and problems were reviewed with special interest given to the humane slaughter act, and whether sufficient funds were available to adequately meet the growing demands on this division.

The second meeting was held in Washington, D. C., at the Agriculture Building on April 26, 1960, and was devoted to meeting with division chiefs
for a discussion of programs, various projects and field studies. In addition, future possible programs were discussed which included a meeting with the Budget Committee of the Agriculture Research Service on the 1962 Budget.

Your Committee expressed its concern over the fact that many of the matters reviewed in previous meetings are yet to be achieved. Namely adequate facilities and personnel along the Canadian border for entry inspection and also the fencing of portions or all of the Mexican border.

Your Committee stressed the need for expanded control of biologic production, because of the increase of production from 2.6 billion to 4.3 billion doses in 1958 to 1959. The Committee therefore strongly urges more funds to increase this inspection. We believe the force of the Animal Inspection and Quarantine Division has long been understaffed to carry out the important responsibility of keeping foreign animal diseases from our animal populations. We would urge that sufficient added funds be obtained to provide for the employment of a more adequate inspection staff.

In the Tuberculosis Eradication Program a small but steady rise in the upward trend in infection is still being noted and the Committee recommended increased funds to be made available so that increased tuberculin testing can be undertaken.

In Brucellosis, concern was voiced by the Committee in regard to the inclusion of a 40 percent-60 percent matching feature by the Senate Agriculture Appropriation Committee. The recommendation of this Committee is that we urge Congress to remove this feature of their recommendation.

At the time of this meeting much concern was felt relative to the possible lowering of the Brucellosis Appropriation Funds from $20 million to $15 million, which as you now know was finally set at approximately $19 million. This increase came about chiefly through the action instigated by the members of this Association.

This Committee also discussed and felt that a Hog Cholera Eradication Program should be pursued as the livestock industry and others concerned are ready for such a program.

It was noted that the 1962 year would inaugurate full scale operation at the National Animal Disease Laboratory and the Committee felt that no less than 3½ million would be required to adequately finance this project, and another one million dollars would be needed for non program or housekeeping costs. The Committee also again stated its previous comments on the need for research work to be pursued on toxic-chemical residues and that not less than ½ million be allocated for this program. We have asked and the Department has indicated approval of the conversion of the Beltsville facilities to Parasite Research. We urge that $3 million be sought to get this job started in fiscal 1962. Parasite problems are enormous and have a direct bearing and relationship to disease problems of a non-parasitic nature. This, too, is urgent.

With regard to the Meat Inspection, this division continues to grow in rapid bounds due to population increases and new frozen food products being placed on the market. Funds appropriated seem to be adequate at this time.
A third meeting of the Committee took place at the American Veterinary Medical Association meeting at Denver on August 16, 1960. At this meeting the various Division Heads of Animal Disease Eradication Division, Dr. C. D. Van Houweling, Assistant Administrative Officer and Dr. C. H. Pals, Director, Meat Inspection Division, met with the Committee, and we discussed the various programs including programs and results that had come about since our earlier meeting in April in Washington, D. C.

In addition to the present programs, Doctor Van Houweling went over the present status of the two epizootics, African Swine Fever and African Horse Sickness now raging in Africa and the European Continent.

It should be noted that this Committee met on April 24, 1960, with the Executive Committee of the National Association of State Departments of Agriculture, which was prior to our meeting with the Agriculture Research Committee. This request was from the officers of the National Association of State Departments of Agriculture, and I do not know at this time whether this will become an established practice. Much can be attained by this close liaison and I feel that we should look forward to this type of meeting whenever possible.

The fourth meeting was held at Charleston, West Virginia, October 19, 1960.

As Chairman of this Committee, I wish to take this opportunity in thanking the various Committee members and members of the Staff of the Agriculture Research Service, for their valuable assistance and cooperation during the year. As a member of this Committee since 1954, with the exception of 1957, it has been possible to evaluate the great progress that has come about during those years, and also be cognizant of the various problems that yet lie before the Association. I do feel that it is of tremendous value for Committee members to be able to be on this Committee and also be the officers of the Association as in this fast day and age, coupled with the regular duties of each of the officers, they need to be informed and understand the programs and goals of the Agriculture Research Service throughout the years rather than on a yearly appointive basis.

Therefore in conclusion, it is my opinion that the action of the Executive Board of this Association in combining the Advisory and Legislative Committees into the Committee on Federal Programs and Policies, and that these members be the officers of the Association, has proven to be a step in the right direction in strengthening not only the Association, but making it possible for your officers to better serve you.

A complete file of the 1959 and 1960 minutes has been established, and it is suggested that this be continued and passed on to each successive chairman so that a complete detail file will be established for future committees.
AMENDMENT TO THE CONSTITUTION

Dr. J. G. Milligan: Last year at our meeting in San Francisco, we offered an amendment to the constitution of this Association making the work of the organization a little more easy and efficient should we adopt this amendment.

The amendment merely changes the officers of this Association from President, First Vice-President, Second Vice-President, and Third Vice-President, to President, President-Elect, First Vice-President and Second Vice-President. It in no way changes the duties of the President, but merely changes the duties heretofore assigned to the First Vice-President to the President-Elect, and the duties assigned to the Second Vice-President to the First Vice-President, and those duties assigned to the Third Vice-President would now be assigned to the Second Vice-President. There would be no position known as Third Vice-President.

This amendment has been published in your 63rd Annual Proceedings and has been approved by the Executive Committee. It now awaits the action of this body. Since you have all read it in the proceedings, I will not read it into this one.

I move, Mr. Chairman, that this proposed amendment be adopted by this Association.

Dr. G. H. Good: I second the motion.

Chair: The motion has been made that this amendment be adopted and you have heard the second. Is there any discussion?

All those in favor of this amendment to the Constitution will please signal by saying “Aye.” Opposed, “No.”

The Chair declares the amendment adopted.
COMMITTEE ON RESOLUTIONS

A. L. Brueckner, Chairman, Baltimore, Maryland; F. G. Buzzell, Augusta, Maine; T. C. Green, Charleston, West Virginia; R. W. Smith, Concord, New Hampshire; R. L. West, St. Paul, Minnesota.

The following resolutions are presented for your consideration and action:

RESOLUTION NO. 1 — FUNDS FOR FEDERAL-STATE BRUCELLOSIS ERADICATION PROGRAM

WHEREAS, the accelerated brucellosis eradication program has made rapid strides in some areas of the United States; and

WHEREAS, there is need for continued intensive effort to protect the gains made, so far, and to carry the work to a successful conclusion;

NOW, THEREFORE, BE IT RESOLVED, that the Executive Committee of the United States Livestock Sanitary Association, assembled in Convention in Charleston, West Virginia, October 18-21, 1960, request the Congress of the United States to continue the appropriation of the United States Department of Agriculture for the program, at least at the same level as applies in the 1961 fiscal year; and

BE IT FURTHER RESOLVED, that copies of this recommendation be forwarded by each State Regulatory Official to his Senator and Congressman and to the Secretary of Agriculture.

RESOLUTION NO. 2 — SHEEP SCABIES

WHEREAS, the number of flocks of sheep reported as affected with psoroptic scabies is unnecessarily large; and,

WHEREAS, the United States Department of Agriculture has attempted a speed-up of the sheep scabies program within the past years; the program is moving too slowly; and there appears little likelihood of a complete eradication within the United States for many years to come; and,

WHEREAS, we do possess the organization and the technical understanding for a complete eradication of the infestation; therefore,

BE IT RESOLVED, that the Executive Committee of the United States Livestock Sanitary Association, assembled in Convention at Charleston, West Virginia, October 18-21, 1960, request the Secretary of Agriculture to develop a federal-state cooperative project for the eradication of sheep scabies with sufficient scope and intensity to achieve complete eradication not later than January 1, 1964; and

BE IT FURTHER RESOLVED, that a copy of this Resolution be sent to the Secretary of the United States Department of Agriculture.

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RESOLUTION NO. 3—SCRAPIE RESEARCH

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

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

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

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

BE IT RESOLVED, that the Executive Committee of the United States Livestock Sanitary Association, assembled in Convention at Charleston, West Virginia, October 18-21, 1960, request the Secretary of the Department of Agriculture to increase the contribution of personnel and funds to the research projects of the British research institutions, as the most economical and effective way to accelerate the production of results from scrapie research; and

BE IT FURTHER RESOLVED, that a copy of this resolution be sent to the Secretary of the United States Department of Agriculture.

RESOLUTION NO. 5—CONTROL OF FOREIGN SHEEP REACHING THE WEST COAST OF THE UNITED STATES

WHEREAS, there have been thousands of foreign sheep brought to West Coast ports in the last two years; and

WHEREAS, the present system of quarantine and inspection has prevented the introduction of serious sheep diseases;

THEREFORE BE IT RESOLVED, that the Executive Committee of the United States Livestock Sanitary Association, in Convention assembled at Charleston, West Virginia, October 18-21, 1960, commend the Disease Regulatory Officials involved for their insistence upon stringent health examinations of imported sheep and their successful efforts, to date, to keep these imported foreign sheep in the category of “for slaughter only.”

RESOLUTION NO. 6—ESTABLISHMENT OF A VETERINARY BRANCH IN THE INTERNATIONAL COOPERATION ADMINISTRATION

WHEREAS, the International Cooperation Administration of the Department of State of the United States has and is presently engaged in veterinary programs in many foreign countries, and

WHEREAS, these veterinary programs involve expenditure of considerable amounts of money for material, technical assistance, and training of veterinary personnel in this and other countries, and

WHEREAS, such programs involve highly technical duties and decisions, and

WHEREAS, the International Cooperation Administration employs veterinarians in groups and singly for duty in several foreign countries, and
WHEREAS, such veterinary activities of the International Cooperative Administration are presently primarily the responsibility of the Livestock and Livestock Products Branch, and

WHEREAS, the International Cooperation Administration does not and has not in the past employed permanent veterinary personnel on its Washington Headquarters Staff, and

WHEREAS, there is no over-all technical guidance, evaluation and coordination of veterinary programs, and

WHEREAS, such over-all technical guidance, evaluation and coordination of veterinary programs can be effectively accomplished only by qualified veterinary personnel, and

WHEREAS, such work is of markedly vital and important nature both to foreign countries and the United States, and

WHEREAS, the American Veterinary Medical Association at its last annual Convention has passed a resolution recommending to the Department of State that a Veterinary Branch be established in the International Cooperation Administration to implement, execute and coordinate all veterinary activities of the International Cooperation Administration:

THEREFORE, BE IT RESOLVED, that the United States Livestock Sanitary Association does, in Convention assembled in Charleston, West Virginia, October 18-21, 1960, endorse the principles set forth in the aforementioned resolution of the American Veterinary Medical Association and recommends to the Department of State that it establish a Veterinary Branch to implement, execute and coordinate all its veterinary activities for the purpose of furthering more effective mutual assistance among all countries involved or to be involved in the International Cooperation Administration programs, and

BE IT FURTHER RESOLVED, that a copy of this resolution be sent to the Office of International Affairs of the State Department, and to the Planning and Operation Office of the International Cooperation Administration.

RESOLUTION NO. 7—CHARLESTON MEETING

WHEREAS, the Sixty-fourth Annual Meeting of the United States Livestock Sanitary Association, in Convention assembled at Charleston, West Virginia, October 18-21, 1960, has proven to be one of the most enjoyable and fruitful meetings of the Association, and

WHEREAS, arrangements by our genial and capable host, Dr. T. C. Green, State Veterinarian of West Virginia, and his staff, and Dr. Allen S. Barnes, for hotel accommodations and entertainment, have been both enjoyable and profitable to all members of the Association,

THEREFORE, BE IT RESOLVED, that the members of the Association express their sincere appreciation for courtesies extended by the Union Carbide Company, including the delicious refreshments and tour of the Research Center and the interesting discussion given by officers of the Union Carbide; to the Charleston Chamber of Commerce, the Daniel Boone Hotel, Woodrums
RESOLUTIONS

Furniture Company, Governor Cecil Underwood and Commissioner of Agriculture John T. Johnson, the press and radio, National Band and Tag Company, all of whom contributed so much to the comfort and enjoyment of our members of the Sixty-fourth Annual Meeting of the United States Livestock Sanitary Association, and

BE IT FURTHER RESOLVED, that copies of this resolution be sent to the appropriate individuals concerned.

I move that this report be referred to the Executive Committee.
REPORT OF THE COMMITTEE ON DISEASES OF SHEEP AND GOATS


The Committee on Transmissible Diseases of Sheep and Goats was formed because the officers of the Association believed more study should be devoted to some of the diseases that create regulatory problems with these species of animals.

It is the desire of the Committee to commend last year's Committee report to you for study and reference. The diseases referred to, foot rot, scrapie, bluetongue, ovine virus abortion, epididymitis, scabies, and pedunculosis are currently important and everything possible should be done to control them. We wish to reemphasize the recommendations and resolutions presented to you and approved by your Association last year, with special emphasis on Resolution No. 3, and specifically request this resolution be sent to the Secretary of Agriculture.

The intent of this report is to call your attention to several additional transmissible diseases that are of real importance and which in some areas are little understood. An understanding of the etiology, and immunology is important, in so far as that information is available, since such an understanding is vital to the proper regulatory handling of the conditions.

CONTAGIOUS ECTHYMA

Sore mouth (contagious ecthyma, or contagious pustular dermatitis) is an acute specific infectious disease of sheep and goats caused by the virus Borrelia ecthymatis. It is characterized by lesions progressing from papular to vesicular to pustular to scab formation on the epithelial layers of the skin and certain of the mucus membranes. In addition to sheep and goats, man is susceptible to the virus, by infection and by allergic reaction from handling of the virus.

The virus is extremely resistant to weather changes and may infect a premise for years. The disease is common on the grazing ranges of the western United States with the heaviest concentration in the southwest. It is transmitted by contact with infected animals or with the scabs from previously infected animals. Lambs and kids are the most susceptible. Older animals may develop a light form of the disease if vaccinated too young. However, very severe lesions may follow infection of older animals provided they have had no previous contact with the infection.
The incubation period is approximately four days, and the disease will pass through the three phases of papular to vesicle to pustular in approximately five to 11 days following exposure. The scab will usually fall off in about 25 to 30 days depending on the weather. Hot, dry weather tends to allow the scab to fall sooner than moist, cold weather.

Recovery from a natural infection is followed by permanent immunity, as is the case using a live virus vaccine. Cross immunity tests have shown that strains of Borrelia ecthymatis are homologous for all practical purposes in their antigenicity regardless of source.

A differential diagnosis between vaccina, sheep pox, proliferative dermatitis, sore mouth, ulcerative dermatosis and infectious foot-rot is necessary since the etiologic agents are entirely different. The sore mouth vaccine immunization is specific against that virus.

There is a confusing disease condition affecting sheep and goats of the Southwest United States that is classified as contagious ecthyma. However, at the present time there is some question as to etiology. It is similar to ecthyma in appearance, incubation period and infectiousness; it affects old sheep as well as lambs, it apparently will not produce immunity from a natural infection nor from vaccination, lambs and older sheep may have the condition several times a year and finally some similarity exists between ulcerative dermatosis and this condition.

**ULCERATIVE DERMATOSIS OF SHEEP**

The name ulcerative dermatosis of sheep was suggested to include the conditions of venereal infection and lip-and-leg ulceration formerly thought to be caused by different infectious agents and to be separate and distinct diseases. Now, they are known to be caused by the same virus and to be but different manifestations of the same disease. The conditions are infectious
and the lesions, regardless of location, are the same. Usually lip-and-leg lesions or genital lesions are found in the flock. Rarely does one find the lips, legs and genitalia affected in the same animal or among the individuals of the flock.

The lesions are characterized by circumscribed ulceration of the epidermal tissue, affecting the skin of the lips, legs, feet and external genital organs. There are no noticeable early systemic reactions. Probably the first observable symptom would be lameness or urinary disturbance as a result of the lesions that may have gone unobserved and progressed to an advanced stage before recognition. The lesion, regardless of anatomical location, is a circumscribed ulcer with a raw, easily bleeding crater varying in depth and extent, containing an odorless creamy pus but covered from the first with a scab. Face lesions occur on the upper lip between the border of the lip and the nasal orifice and on the chin. The ulcerative process may, in very severe cases, perforate the lip. Feet lesions occur between the coronet and the carpus or tarsus. Posthitis lesions partially or completely involve the preputial orifice and may become so severe as to produce phimosis. The glans penis may become involved with extensive ulceration so that the animal becomes unfit for further service. The lips of the vulva show edema, ulceration and scab formation.

Diagnosis depends entirely upon recognition of the characteristic ulcerative lesion. Differentiation between this disease and contagious ecthyma, a proliferative lesion is fundamental. It is difficult and in some instances nearly impossible without resorting to sheep inoculation to differentiate between non-infectious posthitis and vulvitis and this specific virus infection. There is little likelihood of confusion with foot-rot since it is strictly a process of necrosis. Inoculation of sheep previously immunized against sore mouth will quickly verify the presence of a second virus since there is no cross immunity. Furthermore, a natural infection produces no immunity against subsequent infections.

Infected animals should be isolated. Those having genital lesions should not be used for breeding. Treatment is unsatisfactory. Untreated cases seem to heal as rapidly as the treated ones. Recovery is unpredictable; it may take two to eight weeks. Don't attempt treatment unless: (1) lesions must be healed in time for breeding, (2) lip lesions interfere with eating, (3) foot lesions make animals so lame that they are losing flesh.

If treatment is instituted, remove the scab, clean up the ulcer by removing all dead tissue and then apply any of the following, one seems as effective as the other: silver nitrate, saturated 30 percent copper sulphate, four percent formalin, five percent creosol or sulfa-urea powder. Foot and lower leg lesions can be treated advantageously with copper sulphate or formaldehyde solutions in foot bath troughs.

PNEUMONIA IN LAMBS

Some helpful information has been developed during recent years on one of our little understood lamb-disease complexes. It has been found that the
condition known as shipping fever, pneumonia, summer pneumonia and various other terms probably constitutes at least two diseases. It seems appropriate to offer some clarification at this time regarding the etiology.

Very briefly an inapparent pneumonia caused by a member of the psittacosis-lymphogranuloma group of viruses occurs in the young lamb. This infection produces small areas of consolidation in the anterior ventral portion of the lung which become the initial site of a secondary bacterial pneumonia later in life when the lamb is stressed by such management procedures as weaning, shipping and feed changes.

Attempts at improved methods of control of this disease-complex have been largely centered on treating affected animals. This has resulted in better management of an infected flock with reduction in death loss, weight loss, and reduced convalescent period. However, very little progress has been made in methods of prevention which is, of course, fundamental to a sound control program. Since this is one of the most costly problems with which the sheepman is faced, the Association should assume some responsibility for the promotion of additional research on the problem pointed at an effective prevention program.

**SCRAPIE**

Additional scrapie knowledge is of the utmost importance to the sheep industry of the United States. Resolution No. III of last year requested the Secretary of the United States Department of Agriculture to accelerate research on the project. It is our understanding that the work has been increased. However, we feel that a greatly accelerated program should be initiated immediately.

**SCABIES**

Your Committee believes that since we do have the necessary information for a complete national eradication program available, eradication should be our immediate goal. This toleration of an eradicable disease such as scabies, borders on a national disgrace. Since the knowledge is at hand for an eradication program, your Committee urges the state and federal animal disease control authorities to institute an all-out, greatly accelerated program that will completely eradicate this condition within the next two years. They also recognize that increased state and federal funds are essential to carry out this program on an effective level, and that the request for sufficient funds must come from the sheep industry, we therefore recommend that the problem of extra funds be brought to the attention of industry in each state and finally to the elected state and federal representatives of each state by industry.
REPORT OF REPRESENTATIVES TO THE ANNUAL MEETING OF
NATIONAL ASSOCIATION OF STATE DEPARTMENTS OF
AGRICULTURE

T. J. GRENNAN, JR., Providence, Rhode Island


The 42nd Annual Convention of the National Association of State Departments of Agriculture was held at the Brown Palace Hotel, Denver, Colorado, September 4-8, 1960. Representatives from the United States Livestock Sanitary Association attending this meeting were: Dr. J. A. Hay, Chicago Illinois, and Dr. T. J. Grennan, Jr., Providence, Rhode Island.

The delegates from this Association were invited to attend all committee sessions, and were specifically requested to attend and to participate in the proceedings of the Committee on Animal Health. The majority of the resolutions considered by this Committee were not adopted. As a result of careful screening and considerable deliberation, six resolutions were adopted by this Committee, and were unanimously passed by the governing body. These resolutions follow:

RESOLUTION NO. II—MASTITIS

WHEREAS, mastitis is now the number one destroyer of the health of the nation's dairy cattle, which fact is, on one hand, a tribute to the progress that has been made in the prevention, control and eradication of more damaging diseases, and, on the other hand, a sad commentary upon our lack of vision; and

WHEREAS, mastitis is well known to all who have even a casual relationship with dairying; the complexity of the causes, the difficulties of prevention and treatment, and the frustrations from a lack of scientific knowledge are all too well known to dairymen. Consequently, much is said and written about this condition, but not nearly enough is being done about it.

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture, assembled in convention at Denver, Colorado, September 4-8, 1960;

I. Take the lead in a twofold program;
   a. To greatly increase the quality and quantity of basic research on all aspects of the mastitis problem by colleges of veterinary medicine, state agricultural experiment stations, industry, and the Agricultural Research Service.
   b. To provide in each state, according to need, laboratory assistance to dairymen and veterinarians in the diagnosis and identification of the causes of cases of mastitis.

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II. Request the Executive Committee of the National Association of State Departments of Agriculture to bring this matter forcibly to the attention of local, state and national organizations of the dairy industry for the purpose of aiding in the accomplishment of these objectives; and

III. That a copy of this resolution be sent to the Secretary of Agriculture, United States Department of Agriculture, urging that a committee be formed to study the objectives and report their findings to the Agricultural Research Service; and

IV. That a copy of this resolution be sent to the United States Livestock Sanitary Association and that the organization be urged to form a mastitis committee to work with the United States Department of Agriculture to accomplish this objective; and

BE IT FURTHER RESOLVED that each state present briefly its mastitis control program to the Executive Committee of the National Association of State Departments of Agriculture for review and study.

RESOLUTION NO. II—INTERSTATE SHIPMENT OF SWINE

WHEREAS, heavy farm losses continue to occur as a result of the interstate traffic in swine which are infected with or exposed to disease, particularly in swine sold as "feeder pigs"; and

WHEREAS, many of the states do not have effective regulatory and enforcement procedures to curb this illicit traffic in swine;

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture, assembled in convention at Denver, Colorado, September 4-8, 1960, urge the development of uniform laws and regulations governing the movement of both feeding and breeding swine and their adoption and enforcement by each of the states;

BE IT FURTHER RESOLVED, that this Association request the Executive Committee of the National Association of State Departments of Agriculture to take cognizance of this problem and to assist in the development and adoption of uniform regulatory and enforcement procedures by its member states.

RESOLUTION NO. IV—TUBERCULOSIS RESEARCH AND ERADICATION APPROPRIATION

WHEREAS, the incidence of Bovine Tuberculosis has materially increased in some states; and

WHEREAS, the number of animals tested must be materially increased if this disease is again brought under control; and

WHEREAS, there appears to be many unknown factors involved in the increase in incidence of the reaction, the answers to which can be found only through research;

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture, assembled in convention at Denver, Colorado, September 4-8, 1960, commend the United States Department of Agriculture
for the research it is conducting in cooperation with the University of Wisconsin and the Michigan State University in an attempt to find answers to these unknown factors; and that the above mentioned research being conducted jointly by the United States Department of Agriculture, the University of Wisconsin, and Michigan State University be continued until satisfactory solution to these unknown factors have been found; and

Be It Further Resolved, that this Association request its Executive Committee to take whatever action necessary to secure a minimum annual $3,000,000 federal appropriation for tuberculosis eradication programs.

RESOLUTION NO. V—PROHIBIT INTERSTATE MOVEMENT OF HOG CHOLERA VIRUS

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

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

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

Now, Therefore, Be It Resolved, by the National Association of State Departments of Agriculture assembled in Convention at Denver, Colorado, September 4-8, 1960, that its Executive Committee urge the United States Secretary of Agriculture to prohibit the movement and sale between the various states of swine which have been immunized with virulent hog cholera virus.

RESOLUTION NO. VI—HOG CHOLERA

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

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

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

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

Now, Therefore, Be It Resolved, by the National Association of State Departments of Agriculture assembled in convention at Denver, Colorado, September 4-8, 1960, that its Executive Committee urge those states which have not passed legislation prohibiting the sale of virulent hog cholera vaccine to do so as soon as possible, and to also enact legislation requiring the use of modified live virus hog cholera vaccine as the method for immunizing swine against hog cholera.
RESOLUTION NO. VII—BRUCELLOSIS ERADICATION APPROPRIATION

WHEREAS, the brucellosis program conducted cooperatively between the several states and the United States Department of Agriculture has demonstrated that this disease can be brought under control and eventually eradicated if the current program is diligently pursued at the accelerated rate; and

WHEREAS, it can be shown that all of the states can reach a modified certified status by 1966 if federal appropriations are at a $20 million level; and

WHEREAS, any lesser appropriation will result in a delay in the remaining states reaching a modified certified status and will cost many, many millions of dollars more;

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture, assembled in convention at Denver, Colorado, September 4-8, 1960, request its Executive Committee to take whatever steps are necessary to secure a minimum federal appropriation of $20,000,000 annually.

A seventh resolution, which was acceptable by the members of the Committee on Animal Health, was added as a companion resolution in the Committee on Plant Industry. In order to avoid duplicate resolutions, the Committee of Animal Health deferred to the Committee on Plant Industry. This resolution follows:

RESOLUTION NO. XII—APPROPRIATIONS FOR PLANT AND ANIMAL PORT INSPECTIONS

WHEREAS, the foreign plant quarantine service of the United States Department of Agriculture, Agricultural Research Service, Plant Quarantine Division, constitutes this nation’s first line of defense against the introduction of new insect pests and plant diseases of foreign origin which are extremely dangerous to crops in this country; and

WHEREAS, the rapid increase in travel and commerce from foreign countries, indelibly associated with the recent opening of the St. Lawrence Seaway and other border ports of entry, as well as, with the establishment of numerous new international airports throughout this land, have increased exceedingly the plant pest risks, and

WHEREAS, the rate of expansion of the Plant Quarantine Division has been insufficient to meet the greater service responsibilities arising from this cause,

THEREFORE BE IT RESOLVED, that the National Association of State Departments of Agriculture in annual meeting at Denver, Colorado, September 4-8, 1960, go on record as recommending to the Congress an increase of $1,000,000 in the appropriation, over the amount appropriated for this service in fiscal year 1961, for use of the Plant Quarantine Division in fiscal year 1962, and
BE IT FURTHER RESOLVED, that the Association take recognition of the dangers of insects and diseases entering this country which affect animals and instruct its Executive Committee to take appropriate action to secure adequate funds for animal inspection and quarantine work.

One of the highlights of this convention was a panel composed of four commissioners representing each of the four regions of the country and a moderator. The topic for this panel was Problems in Agriculture. One of the chief problems as discussed by representatives from each of the regions was the increasing difficulty in gaining legislative and financial support of agricultural problems and for agricultural programs throughout the country. This same difficulty is also applied to all branches of the livestock industry.

A total of 21 resolutions was passed by this Association, seven of which dealt with livestock diseases and control. This number attests to the importance which those that are engaged in the direction of Agriculture place upon the livestock industry.
This Committee met and the following recommendations were approved by the members present:

(1) This Committee recognizes the progress made by the Animal Inspection and Quarantine Division of the Agricultural Research Service in effecting improved sanitary facilities at ports of entry along the Canadian border. It is recommended that these efforts be continued. These facilities should include shelter from inclement weather, inspection chutes, satisfactory pens, and cleaning and disinfection equipment. The installation should compare favorably with public stockyards and specifically approved livestock markets.

(2) It is reported that in many instances Canadian livestock released at the border, consigned to points in the United States, fail to arrive at destination. It is urged that the livestock official of the state involved promptly advise the Animal Inspection and Quarantine Division of the non-receipt of such shipment so that it may be traced and a recurrence of the incident prevented.

(3) While considerable improvement has been noted in some states, during the past year, in the issuance of permits for the admission of livestock, it is recommended that a continued effort be made to have state officials furnish permits promptly and limit the telegrams authorizing the shipments to the fewest possible number of words in order to reduce the cost.

(4) It is recommended that a study be made in an effort to reduce death losses in stocker and feeder pigs. It is further recommended that the handling of such swine be more strictly controlled so as to prevent their movement from market to market with the resulting loss of identification and duplication of vaccination.

(5) In order to expedite the handling of official vaccinates and cattle from certified areas through stockyards and auction markets it is recommended that some acceptable uniform procedure be established whereby these animals may be identified and certified to with the least possible delay.
(6) The receipt of sheep affected with contagious ecthyma (sore mouth) continues to be a problem at some public stockyards. It is recommended therefore that if the necessary legal authority becomes available, the Animal Disease Eradication Division and the appropriate state livestock official agrees to an acceptable procedure which will permit the prompt handling of such animals.
AN IMPROVED LEPTOSPIRA POMONA BACTERIN

LEE F. SCHUCHARDT, M.A.,* GEORGE E. BRIGHTENBACK, D.V.M.,† AND JAMES E. PRIER, D.V.M., PH.D.‡

In recent years, the economic importance of leptospirosis in livestock has been well established. The disease has been repeatedly diagnosed and confirmed serologically in both cattle and swine (1-3) and apparently is present in every geographic region of the United States (4). Experience in practical application and controlled field trials (5, 6) show that Leptospira pomona bacterins are effective in limiting the spread of leptospirosis. Several workers have demonstrated that bacterins (7-12) and a soluble antigen derived from cultures of the organism (13) produce immunity.

The early bacterins described by York and Baker (5, 6) were prepared from leptospira grown in embryonated eggs. Brown, et al (7) demonstrated that bacterins grown in culture medium were of greater antigenicity than egg grown bacterins. Because of the superior antigenicity and ease of handling most commercial bacterins are prepared using Stuart's medium (14) with approximately 10 percent inactivated hemolyzed rabbit serum.

Recent reports from the field (15-17) have indicated an increasing number of anaphylactoid reactions following the use of L. pomona bacterins. These reactions have been reported following the use of vaccine from several manufacturers. The over-all reaction rate appears to be two to four percent, however, in individual herds the rate may vary from zero to 40 percent of the animals vaccinated. In an article on leptospirosis Binkley (18) gives a direct warning in regard to anaphylactic reactions, stating definitely that rabbit serum is the cause.

The purpose of this paper is to describe an improved bacterin** essentially free of rabbit serum and to furnish evidence of its safety and effectiveness.

MATERIALS AND METHODS

Preparation of Bacterins. The method of preparation of the regular bacterins used in these studies was essentially that described by Brown, et al. (7). The T 262 strain of L. pomona was grown for 10-14 days at 30-32°C in a modification of Stuart’s medium made according to directions obtained from the Army Medical Service Graduate School. The organisms were killed by

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† Animal Science Research, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey.
‡ Present address: School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
** “Anti-lepto”—Leptospira pomona bacterin prepared by the Biological Production Laboratories, Merck, Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pennsylvania.
the addition of thimerosal in a final concentration of 1:10,000. In the improved bacterins the cells were freed of rabbit serum and adjusted to a final concentration of 2,500 million organisms per ml. The final rabbit serum content was less than $2.4 \times 10^{-6}$ percent in contrast to the whole culture bacterins that contain up to 10 percent rabbit serum. The recommended dose was two ml.

**Potency Test for Bacterins in Guinea Pigs.** For the purpose of comparing different lots of bacterin, a guinea pig test (8) was used in which guinea pigs were inoculated with dilutions of bacterin followed by challenge with virulent leptospira. Guinea pigs weighing 350-400 grams were injected in the foot pad of the right hind leg with one ml. of the test bacterin. At the same time a suitable number of control animals were set aside. All guinea pigs were kept in an air-conditioned room and temperatures were taken and recorded daily for five days prior to challenge. Fourteen days after vaccination all animals were challenged by intraperitoneal injection of one ml. of defibrinated blood from a pool of bleedings obtained from *L. pomona* infected guinea pigs. Individual temperatures were taken daily for 10 days. Any animal showing a febrile response of $104^\circ F.$ or more for two or more consecutive days was considered an infected animal provided the febrile response occurred 48 hours subsequent to challenge.

**Potency Test for Bacterins in Hamsters.** Groups of 10 hamsters of one sex, weighing 100-125 grams, were injected intraperitoneally with 0.5 ml. of graded dilutions of the test bacterin. A standard reference bacterin was included with each test. Seven days after vaccination test animals were challenged by an intraperitoneal injection of 0.25 ml. of a 1:5 dilution of pooled blood from hamsters infected with a virulent strain of *L. pomona*, known as the MLS strain, obtained from Dr. James A. Baker of Cornell University. Additional groups of 10 normal hamsters were injected at the time of challenge with the challenge dose and graduated dilutions of the challenge dose. The animals were observed daily for 14 days and the number of deaths recorded. The $ED_{50}$ and relative potency of the test bacterins were calculated by the method of Wilson and Worcester (19-22).

**Anaphylactogenicity Test.** Guinea pigs weighing approximately 350 grams each were injected subcutaneously with 0.1 ml. of the test bacterin. After 21 days the animals were challenged intravenously with 0.5 ml. of the same bacterin. The animals were observed immediately for symptoms of anaphylaxis. For a vaccine to be considered satisfactory, all of the animals must survive and must not show significant symptoms of anaphylaxis (i.e., coughing, convulsions, or death).

**Animal Safety Test.** Animal safety tests were performed using mice and guinea pigs as recommended by the NIH for Pertussis Vaccine (23). Each of three guinea pigs, weighing 300-400 grams, was injected intraperitoneally with five ml. of the bacterin under test. Each of two white Swiss mice, weighing 17-21 grams, was injected intraperitoneally with 0.5 ml. of the
vaccine under test. All animals were observed for seven days. For a vaccine to be considered satisfactory, all the animals must survive and must not show either significant symptoms or loss in body weight.

EXPERIMENTAL RESULTS

Sensitivity to Rabbit Serum in Cattle. A calf, approximately three days old, was obtained from one of the herds that had experienced anaphylactoid reactions following use of leptospirosis bacterins. The dam of this calf had reacted. Two-tenths milliliter of *L. pomona* bacterin was injected intradermally at a point approximately one inch below the angle of the mandible in the right side. The same amount of rabbit serum was given at the same point on the left side. A reaction, characterized by a hard swelling that was mobile, and about one to 1½ inches in diameter, occurred at both sites within 30 minutes. Intradermal injections of "serum-free" leptospira cells or sheep serum, given at the same time in other sites of the neck, did not elicit a reaction.

Attempts to demonstrate passive anaphylaxis in guinea pigs using serum from the above calf and either whole bacterin, "serum-free" cells, or rabbit serum were unsuccessful.

Active Anaphylaxis in Guinea Pigs. Skin sensitivity tests in calves indicated that the rabbit serum in *L. pomona* bacterins was responsible for the reactions seen in cattle. The following experiment was run to further clarify this point. Groups of guinea pigs, each weighing 300-350 grams, were injected subcutaneously with 0.1 ml. of either leptospira bacterin, normal rabbit serum, or "serum-free" leptospira cells. After a 21-day interval groups of animals were challenged intravenously with a shock-inducing dose of 0.5 ml. of one of the antigens. The results are summarized in Table I. Both the leptospira bacterin, that contains rabbit serum, and normal rabbit serum were able to sensitize and to shock guinea pigs. The "serum-free" cells were unable to either sensitize or shock guinea pigs. These results indicate that rabbit serum is the agent responsible for anaphylaxis in guinea pigs.

Potency Tests of Improved Bacterin. Since the washed leptospira cells did not sensitize guinea pigs or cause reactions when injected intradermally into a sensitive calf, it was of interest to determine whether bacterins freed of rabbit serum would pass the potency test. The "serum-free" bacterin described above and its parent whole culture bacterin were tested for potency in guinea pigs. Six guinea pigs were retained as controls. The results are summarized in Table II. At dilutions of 1:4 and 1:16 both the "serum-free" bacterin and the original whole culture bacterin protected six of six guinea pigs. Five of six control guinea pigs showed characteristic febrile reactions. This test indicates satisfactory potency of both bacterins.

The "serum-free" bacterin was tested for potency in hamsters as described above. In addition the parent whole culture bacterin and a standard lyophilized reference bacterin also were tested. The results of these tests are
summarized in Table III. They demonstrate that there is no significant difference between the potency of the whole culture bacterin and the “serum-free” bacterin. Both bacterins show an adequate degree of protection.

*Immunization Studies in Cattle.* Several lots of the “serum-free” bacterin were prepared, and after satisfactory completion of potency, sterility, animal safety, and freedom from anaphylactogenicity tests the bacterins were tested in cattle. No anaphylactic reactions have been experienced in over 2,000 cattle vaccinated with this product. In 26 herds involving approximately 1,150 head that were vaccinated in at least one previous year with some form of leptospirosis bacterin, no evidence of anaphylaxis of any degree has been observed. During this period the same investigators have observed a five percent incidence of strong allergic reactions in cattle revaccinated with the regular type bacterins.

One herd of 39 Jersey cows was vaccinated with the improved bacterin. No evidence of anaphylactic reactions was observed, although reactions were experienced in this herd the previous year following vaccination with the regular type bacterin. The animals were bled immediately preceding vaccination and again one and four weeks after vaccination. Microscopic agglutination tests for *L. pomona* antibody were conducted on the sera. The geometric mean antibody titers for the pre-vaccination and one- and four-week post-vaccination sera were 1:2, 1:16, and 1:18 respectively. The results are summarized in Figure 1. On this spot chart the pre-vaccination

**Figure 1**

*L. POMONA ANTIBODY TITERS - BRYAN HERD*

![Spot chart showing L. POMONA antibody titers for Bryan Herd](image)
titers are plotted on the X axis or abscissa and the highest post-vaccination titers are plotted on the Y axis or ordinate. Most of the dots fall above the diagonal line indicating an increase in antibody titer following vaccination. The 10 animals without demonstrable antibody titers were heifers without previous contact with leptospirosis bacterins.

Another herd of 30 Holstein cows was vaccinated with the improved bacterin. This herd had been previously vaccinated with the regular type bacterin and also had been exposed to leptospirosis. No evidence of allergic reactions of any degree was observed. The animals were bled immediately preceding vaccination and again one week after vaccination. Microscopic agglutination tests for \textit{L. pomona} were conducted on the sera. The geometric mean antibody titers of the pre- and post-vaccination sera were 1:7.1 and 1:23.1 respectively. The individual results are presented in Figure 2. Again most of the data fall above the diagonal line indicating an increase in antibody titer following vaccination. The eight animals without demonstrable antibody titers were heifers without previous contact with leptospirosis bacterins.

Fifty animals in five herds were revaccinated with the improved bacterin 90 days after the initial vaccination procedure. No acute reaction occurred in any of the animals. The Dairy Herd Improvement Association milk tester was testing one of these herds the evening and morning following this vaccination procedure. Therefore an excellent opportunity was afforded for checking milk production. The Dairy Herd Improvement Association milk
weights for these animals are listed in Table IV. The evening and morning difference is within the normal range of variation and, according to the milk tester, showed no indication of any influence of the vaccine on milk production.

DISCUSSION

The observations given in this paper seem to confirm the opinion of Binkley (18) that rabbit serum is the cause of anaphylactic reactions in cattle revaccinated with regular *L. pomona* bacterins. In these studies we demonstrated that the calf of a vaccinated animal was sensitive to rabbit serum, but not to "serum-free" leptospira organisms. It was also shown that regular *L. pomona* bacterin and normal rabbit serum were able to sensitize guinea pigs and to shock previously sensitized guinea pigs. "Serum-free" leptospira organisms were unable to either sensitize or to shock previously sensitized guinea pigs. It is believed, therefore, that the rabbit serum in regular bacterins is responsible for the reactions observed in the field.

Further evidence for this belief is the fact that in over 2,000 cattle vaccinated with an improved *L. pomona* bacterin essentially devoid of rabbit serum, no allergic reactions of any degree have been observed. During the same period the same investigators have noted a five percent incidence of strong allergic reactions in cattle revaccinated with the regular type bacterins. Another measure of the safety of the improved bacterin was the demonstration of no significant differences in milk weights of cows revaccinated with the improved bacterin.

The antibody titers of cows immunized with the improved bacterin compare favorably with those reported by Brown, et al. (7) and are higher than those produced by bacterins grown in embryonated eggs (5, 7). Some heifers without previous contact with leptospira bacterins showed no detectable antibody titers. This is not necessarily an indication of lack of protection, since it has been demonstrated that vaccinated cattle without detectable antibodies are immune to challenge (16, 24, 25). This fact suggests that the *in vitro* agglutination antibody may not be necessarily the one that protects, or at least, that a large amount of antibody is not necessary for protection.

SUMMARY

Rabbit serum in regular *L. pomona* bacterins has been implicated as the cause of anaphylactoid reactions following the use of these bacterins. A vaccine essentially devoid of rabbit serum has been developed. This bacterin does not produce anaphylactic reactions in guinea pigs. Clinical studies in cattle show an adequate response to the bacterin. No anaphylactic reactions were experienced in over 2,000 cattle vaccinated with this improved product.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. E. S. Barclay for the preparation of the improved bacterin and to Mr. Carl Newman and Dr. L. G. Clark for their testing assistance.


### TABLE I

*Active Anaphylaxis in Guinea Pigs*

<table>
<thead>
<tr>
<th>Sensitizing Antigen</th>
<th>Leptospira Bacterin</th>
<th>Shock-Inducing Antigen</th>
<th>“Serum-Free” Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R/T* D/T†</td>
<td>R/T D/T</td>
<td>R/T D/T</td>
</tr>
<tr>
<td>Leptospira Bacterin</td>
<td>13/14 4/14</td>
<td>5/5 5/5</td>
<td>0/10 0/10</td>
</tr>
<tr>
<td>Rabbit Serum</td>
<td>5/8 3/8</td>
<td>8/8 8/8</td>
<td>0/7 0/7</td>
</tr>
<tr>
<td>“Serum-Free” Cells</td>
<td>0/8 0/8</td>
<td>1/9† 0/9</td>
<td>0/9 0/9</td>
</tr>
</tbody>
</table>

* Anaphylactic Reactions/Total Injected.
† Anaphylactic Deaths/Total Injected.
‡ Very Mild Reaction (sneezing only).

### TABLE II

*Guinea Pig Potency Tests of “Serum-Free” Bacterin*

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Bacterin Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:4</td>
</tr>
<tr>
<td>Whole Culture</td>
<td>6/6*</td>
</tr>
<tr>
<td>“Serum-Free”</td>
<td>6/6</td>
</tr>
<tr>
<td>Controls</td>
<td>1/6</td>
</tr>
</tbody>
</table>

* No. Animals Protected/No. Challenged.

### TABLE III

*Hamster Potency Test of “Serum-Free” Bacterin*

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Dilution of Immunizing Dose</th>
<th>Note Results</th>
<th>ED₅₀</th>
<th>Relative Potency With Reference Bacterin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Culture</td>
<td>1:500</td>
<td>0/10†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>7/10</td>
<td>1.72</td>
<td>3.2X (1.6X—6.1X)</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>4/10</td>
<td>1.78</td>
<td>3.4X (1.6X—7.2X)</td>
</tr>
<tr>
<td>“Serum-Free”</td>
<td>1:100</td>
<td>4/10</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>9/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>1:50</td>
<td>3/10</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>8/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virulence Control</td>
<td>1:5 (Challenge Dose)</td>
<td>0/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:500</td>
<td>0/10</td>
<td>1000+LD₅₀’s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:5000</td>
<td>0/5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Survivals/Total Challenged.
TABLE IV

*Milk Weights of Animals Revaccinated With the Improved Bacterin*

<table>
<thead>
<tr>
<th>Animal</th>
<th>30 Days Pre-vaccination p.m.</th>
<th>30 Days Pre-vaccination a.m.</th>
<th>12-24 Hour Post-vaccination p.m.</th>
<th>12-24 Hour Post-vaccination a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terry</td>
<td>14.0*</td>
<td>17.8</td>
<td>9.7</td>
<td>17.8</td>
</tr>
<tr>
<td>Ruth</td>
<td>11.6</td>
<td>14.3</td>
<td>9.2</td>
<td>12.6</td>
</tr>
<tr>
<td>Dora</td>
<td>8.9</td>
<td>10.2</td>
<td>5.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Maiden</td>
<td>11.5</td>
<td>14.9</td>
<td>10.1</td>
<td>13.8</td>
</tr>
<tr>
<td>Kathy</td>
<td>9.6</td>
<td>13.7</td>
<td>9.0</td>
<td>11.8</td>
</tr>
<tr>
<td>Katie</td>
<td>13.2</td>
<td>16.5</td>
<td>9.6</td>
<td>10.8</td>
</tr>
<tr>
<td>Maria</td>
<td>12.9</td>
<td>16.3</td>
<td>12.3</td>
<td>15.5</td>
</tr>
<tr>
<td>Pee Wee</td>
<td>10.8</td>
<td>13.8</td>
<td>9.2</td>
<td>10.5</td>
</tr>
<tr>
<td>Queen</td>
<td>8.4</td>
<td>10.9</td>
<td>7.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Patsy</td>
<td>14.3</td>
<td>16.4</td>
<td>11.7</td>
<td>15.1</td>
</tr>
</tbody>
</table>

* Pounds.

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SEROLOGICAL TESTS AS INDICATORS OF IMMUNITY*

JAMES A. BAKER AND DOUGLAS S. ROBSOX

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Confidence that products offered for immunization will immunize can be assured only by standardized procedures and adequate replication in product testing. In order to provide a reasonable guarantee of efficacy, regulations must require the use of larger numbers of test animals than are presently employed, especially for those products evaluated by animal protection tests. Under existing regulations governing the licensing of distemper vaccines an inferior product which would fail to immunize a third of the dogs vaccinated has exactly a 50-50 chance of passing the standard requirements, and immunity tests conducted by the New York State Diagnostic Laboratory at Cornell University indicate that there are commercial distemper vaccines on the market today which are less than 60 percent effective.

The more stringent licensing regulations which would be necessary to remedy this situation and justify consumer confidence in the quality of biological products appear prohibitive in contrast to the present meager requirements. Approximately 30 times as many test subjects are needed in some of the animal protection tests in order to provide the desired control over vaccine efficacy. Instead of the two vaccinates and two or three controls presently challenged in these tests, some 60 vaccinates and 60 controls are actually needed to provide reliable evidence for high efficacy (1). To minimize the cost of the animal protection tests required in raising these standards on vaccine quality it becomes necessary to seek and to adopt test procedures which can be performed simply and which will measure vaccine efficacy. Serological tests, based upon sound statistical design, appear to be the solution. Such tests measure antibodies that are formed after antigen has been introduced parenterally into an animal. Because pathogenic agents and analogous bacterins or vaccines act as antigens, antibodies induced by them become indicative either of previous disease or of a vaccination procedure.

Immunity, however, can be ascertained only by actual exposure to the test pathogen under circumstances in which appropriate controls sicken while test animals remain healthy. If a serological test can be correlated with the immunity test it becomes an indicator as reliable as actual exposure. Possibilities are presented for consideration.

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SEROLOGICAL TESTS AS INDICATORS OF IMMUNITY

METHODS

The relationship between serological tests and immunity is better understood if there is agreement on the meanings of certain pertinent terms. As they apply to this discussion (2), an antigen is "any substance that, when introduced parenterally into animal tissues stimulates the production of antibodies and, when mixed with that antibody, reacts with it in some observable way." An antibody is "any substance that makes its appearance in the body fluids of an animal in response to a stimulus provided by the parenteral introduction of an antigen into the tissues and, when mixed with that antigen, reacts with it in some observable way." Immunity will be defined as altered susceptibility to pathogenic agents induced by inoculation either of pathogenic agents or analogous bacterins or vaccines acting as antigens to produce demonstrable antibodies. Animals can show altered susceptibility to pathogenic agents without inoculation and subsequent antibody production. This will be called resistance. It may be defined as that physiological state in which cellular capacity to overcome pathogenic agents has been enhanced by a variety of nonantigenic factors, such as species, age, hormonal influence, nutrition, etc. Because demonstrable antibody forms the basis for differentiation between immunity and resistance the latter includes that period after inoculation when antibodies cannot be demonstrated as well as the period after antibodies have disappeared even though a secondary response (anamnestic reaction) persists. In these particular instances, immunity and resistance blend and defy definition. Because products used for immunization act as antigens, if the time interval for measurement of antibodies is specified, then resistance, whether specifically or non-specifically induced, should not render serological tests inoperable as indicators of immunity.

The serological test. By definition, antigens and antibodies must react in some observable way. The way usually chosen is a serological test. Those most frequently used are: precipitation, in which a soluble antigen reacts with antibody; agglutination, in which a particulate antigen reacts with antibody; complement fixation, in which the failure of hemolysis of red cells shows that the antigen-antibody complex has bound complement; lysis, in which antibodies destroy organisms; toxin neutralization by antitoxin, in which flocculation is observed; and virus neutralization in which antibody inactivates virus.

Each serological test should be standardized so that the results are reproducible in any laboratory with competent technical personnel and adequate equipment. In order to do so, an antigen with measured unitage is required. Then, with conditions of the test controlled, it should be possible to determine antibody as the variable factor. Of course, incorporation of a serum with known antibody content would furnish additional control over the entire procedure. In using the serological test as an indicator of immunity it is necessary not only to have good test procedure but information on appearance and persistence of antibody so that tests of serums will have significance. For example, if antibodies do not appear until 14 days after vaccination
then, although resistant and becoming immune, a serological test would fail to indicate this state but 21 days afterward might do so.

Serological test procedures to detect antibodies for bacterial antigens seem to be more variable in specific application than for viruses. In view of the writer’s greater familiarity with viruses, a generalized procedure is offered only for them, although the need of similar procedures for certain bacterial antigens appears equally necessary. It is hoped that the Committee on Virus Review (Research) of the United States Livestock Sanitary Association will consider standardization of serological procedures for viruses and will give their appraisal of the status of the neutralization test for each as an indicator of immunity. The following outline for standardization of virus neutralization tests is suggested for consideration.

**Correlation between serological results and immunity.** No analytical procedure for measuring biological phenomena can be expected to operate with perfect accuracy because uncontrolled variation always characterizes the underlying biological process. Standards of accuracy which take cognizance of such underlying, error-producing variations automatically become statistical standards, requiring something less than 100 percent accuracy and permitting some uncertainty to exist as to the exact level of accuracy. In the present instance, a serological test of immunity should be expected to err occasionally, because the level of antibody required for immunity may be expected to vary to some degree between animals, but only a low error rate can be tolerated if the procedure is to be used in evaluating a vaccine. Errors due to the serological test become compounded with immunization failures in a vaccine test, and so mask the true efficacy of the vaccine. If the error rate falls below five percent the effect upon a test of vaccine efficacy will be negligible from a practical point of view; consequently, a demonstration of at least 95 percent accuracy will be considered an acceptable basis for substituting a serological method for the method of direct challenge as a test of immunity.

The exact level of accuracy of an analytical technic, such as the serum-neutralization test of immunity, may be measured only by testing an infinite number of animals; consequently, some uncertainty must always exist as to whether the technic actually satisfied the specified minimum requirement of, say, 95 percent accuracy. The level of uncertainty, however, is subject to the control of the experimenter, because it depends upon the number of animals used to test the technic, and this dependence can be formulated mathematically.

A five percent level of uncertainty has been adopted in most scientific fields; the experimenter is thus willing to accept odds of 19 to one against an erroneous decision. Employing the mathematical relation between level of uncertainty and number of animals tested, the experimenter may then determine the number of test animals required to guarantee with odds of at least 19 to one that his decision based upon the experimental results will be the correct decision. If 95 percent accuracy is the minimum acceptable for the
NEUTRALIZATION TEST PROCEDURE

Viral disease __________________________ Host ________________________

Serum status: Before exposure __________ After exposure __________ (in days)

Acute phase __________ Convalescent __________

1. Choice of system
   a. Serum constant—Virus variable
   b. Virus constant—Serum variable

2. Choice of indicator system
   a. Animal
   b. Egg
   c. Tissue culture
      (1) Monolayer
      (2) Dispersed cell
      (3) Plaque

3. Specifications for test procedure
   a. Virus
      (1) Strain and passage level
      (2) Amount (Reed-Muench formula) (3)
   b. Serum
      (1) Dilution
      (2) Standard reference serum used at given titer
      (3) Inactivation of serum
         (a) Temperature
         (b) Time interval
   c. Incubation of serum-virus mixture
      (1) Temperature
      (2) Time interval
   d. Incubation indicator system
      (1) Temperature
      (2) Time interval for final results

4. Evaluation as indicator of immunity
   a. Colostral effects
   b. Nonspecific neutralization
   c. Test specificity
      (1) Present
      (2) Absent
   d. Significant titer range
purposes of an immunity test, then an acceptability test should be designed
to guarantee with odds of at least 19 to one that an immunity test which is
truly less than 95 percent accurate will be rejected and, furthermore, that an
immunity test which is highly accurate, say 99.9 percent, will not be rejected.

Various experimental plans can be derived for an acceptance test having
these statistical properties. The simplest plan mathematically is one in which
the total number of animals to be tested is determined in advance; however,
sequential experiments, now widely used in industrial acceptance testing,
have proved to be much more efficient in the sense of requiring fewer test
subjects. In the sequential experiment the sample size is not fixed in advance;
rather, animals are tested singly or in small groups, and after each test the
results are examined to determine whether or not more animals are needed
to make a decision to accept or reject the hypothesis under test. The optimum
decision rule has been formulated mathematically by A. Wald (4).

\[
\text{acceptance number for } n \text{ tests } = \frac{B}{1-a} + n \frac{p_0}{p_1} - \frac{\log(1-p_1)}{\log(1-p_0)}
\]

\[
\text{rejection number for } n \text{ tests } = \frac{1-B}{a} + n \frac{p_0}{p_1} - \frac{\log(1-p_1)}{\log(1-p_0)}
\]

where

100 $p_0$ percent = the "acceptable-quality" level
100 $p_1$ percent = the "tolerance level"

\[ a = \text{the probability of rejecting an immunity test which is 100 } p_0 \text{ percent accurate} \]
\[ B = \text{the probability of accepting an immunity test which is 100 } p_1 \text{ percent accurate} \]

In the present example, these constants have been set at
\[ p_0 = 0.999 \]
\[ p_1 = 0.950 \]
\[ a = 0.05 \]
\[ B = 0.05 \]

so that

\[ \text{acceptance number for } n \text{ tests } = -0.7431 + 0.0127 n \]
\[ \text{rejection number for } n \text{ tests } = 0.7431 + 0.0127 n \]
For convenience of application, this decision rule may be constructed graphically as shown in Figure 1; animals are then tested in sequence until the number of immunity errors plotted against the number of animals tested falls either in the acceptance or rejection region of the graph.

An acceptance test of a serological technic is a two-phase experiment consisting of two cycles of bleeding and challenging the animals. The first bleeding identifies serologically the animals that are classified as susceptible; these animals are then challenged with the virulent virus, and any failure to produce signs of illness in a serologically susceptible animal constitutes an error. After an appropriate interval of two to four weeks, allowing for the development of antibodies, the animals are again bled and challenged. Because all animals should be immune at the time of this second bleeding, a serum sample which is negative by the neutralization test constitutes an error, unless that animal shows signs of illness following the second challenge. Any failure of agreement between the results predicted by the second bleeding and the results produced by the second challenge counts as an error against the serum-neutralization test.

Acceptance of the serological test by this sequential procedure implies with 95 percent confidence that the true accuracy of the immunity test exceeds 95 percent, for an immunity test which is less than 95 percent accurate stands less than a one in 20 chance of passing the acceptance test (in fact, an immunity test which is truly 97.5 percent accurate stands only a 50-50 chance of acceptance).
Vaccine evaluation by serological test. The process of developing a commercial, live virus vaccine necessarily includes stages at which the product is tested for safety and efficacy (Figure 2), and both of these properties can be ascertained only in animals which are susceptible at the time of vaccination. In an immune animal any vaccine ranging from the unmodified virulent virus to a placebo is both “safe” and “effective” since no signs of illness are produced either by the vaccination or a subsequent challenge. Logically, then, the first step in evaluating a vaccine is the identification and selection of susceptible animals for test vaccination. The only certain means of demonstrating susceptibility in a potential test animal is by direct challenge with

![FIGURE 2. Steps in the Development of a Commercial Vaccine](image)

**Virulent virus**
- Produces illness and productivity loss in natural host.

**Serial passage in alien host**

**Attenuated virus**
- Modified effect in natural host: Produces no signs of illness or produces modified, acceptable signs of illness.

**Preliminary safety test**
- Test of attenuated virus in natural host for signs of illness and transmissibility; if transmissible, then number of passages for reversion to virulence must exceed 5.

**Continued passage in alien host to highest level**

**Determination of minimal immunizing dose**
- Immune response to dosages at the highest passage level to be used.

**Laboratory standardization of vaccine**
- Determination of ID\(_{50}\) for each batch to eliminate batch differences in the commercial dose.

**Efficacy test and safety check**
- Efficacy field tested for acceptability at the highest passage level to be used in vaccine; vaccinated animals observed for signs of illness due to vaccination and checked for immunity in a paired challenge test or unpaired serological test. Repeated annually to assure continued quality.
the virulent agent; this procedure, of course, leaves the animal either immune or dead—in either case, useless as a test subject for vaccine evaluation. We must, therefore, accept some other means of identifying susceptible animals for test purposes and hence must, at the same time, accept some small margin of error in the identification.

Proven serological tests satisfying the requirements noted earlier provide us this almost certain means of identification and greatly simplify the problem of vaccine evaluation, for if the serological test is once accepted as a prevaccination indicator of susceptibility then logical consistency requires that it also be accepted as a postvaccination indicator of immunity. The serological test, when available, thereby substitutes for and completely eliminates the costly direct challenge procedure in a vaccine evaluation program, permitting the efficacy test to be carried out in the field under exactly the same conditions which the commercial vaccine will be required to operate. Detailed plans for a vaccine safety and efficacy test using serological technics are given in two earlier reports (5, 6), and follow the same statistical principles outlined above for testing the accuracy of a serological indicator of immunity.

When no serological test is available the problem of identifying susceptible animals for a vaccine evaluation program is circumvented by using an unvaccinated, control series and a postvaccination challenge of both the vaccinates and controls. With this method the number of vaccinated animals which were naturally immune before vaccination is actually unknown, but can be estimated from the observed number of naturally immune animals in the control lot. The accuracy of this estimate is presumably improved if the vaccinates and controls are littermates; this method of correcting for natural immunity, however, will never be as accurate as the prevaccination serological test and, as a consequence, many more animals may be required to evaluate accurately the quality of a vaccine. A detailed description of this procedure for vaccine evaluation is also given in an earlier report (6) where the advantage of a serological test is made evident by a direct comparison of the two procedures (Figures 3 and 4).

PRESENT STATUS OF KNOWLEDGE

The formal experimental plan of Figure 1 has been applied explicitly to determine the acceptability of a serological test as an indicator of immunity against virus diarrhea (7). In this case, the serum-neutralization test was completely accurate in 59 animals tested. According to the rules of Figure 1, the experiment was therefore terminated at this point and the serological test was accepted as being more than 95 percent accurate. One other serological test, while not subjected to the explicit acceptance procedure described here, has more than adequately satisfied the requirements of Figure 1.

The test in eggs for distemper-neutralizing antibodies in dog serum has been compared with test by virulent virus in over 420 dogs (8), far in excess of the number which would have been required according to the rules of Figure 1. This extensive study of the serological distemper test served
Figure 3. Rule for a sequential acceptance test using paired susceptible vaccinate and contact control animals.

Figure 4. Rule for a sequential acceptance test using triplets of vaccinate, contact control and isolated animals of unknown immunity status.
to reveal the existence of a sigmoid dosage-response relationship between antibody level (dose) and immunity status (response). With distemper there exists a small range of low antibody titers for which immunity status is uncertain. Above this range all dogs are immune and below it all dogs are susceptible; within this range the percentage of immune dogs increases from 0 to 100 percent in a sigmoid manner (9). Test animals in a vaccine evaluation study must therefore exhibit prevaccination titers below this question-able range and the vaccine, if it is to be considered effective, must produce titers above this range. The stochastic dosage-response phenomenon is typical of biological systems and intensive study of other serological tests may be expected to reveal this same phenomenon.

A search of the literature failed to show that any other serological test of immunity has been checked on as many as 59 susceptible animals, though some are well on the way to satisfying the requirements of Figure 1. Burgher (10), for example, reports the successful application of a test in tissue culture for infectious canine hepatitis neutralizing antibodies in the serum of 26 dogs and this test is now being checked on additional dogs. Also nearing completion is an evaluation of the neutralization test in tissue culture as an indicator for immunity to hog cholera (11).

DISCUSSION

As shown in Figure 2, after a virulent virus has been suitably attenuated and the minimal immunizing dose measured at the highest passage level, a particular virus strain can be considered for vaccine. Standardization for vaccine requires assurance by titration of an amount of virus in each batch greater than the minimal immunizing dose and then an efficacy test in animals of the type for which the vaccine is to be produced. Thereafter, if highest passage level is not exceeded, efficacy would seem assured by titration of virus but changes could occur in the vaccine virus to an extent that it no longer immunized, thus invalidating virus assay for efficacy. In order to detect changes, annual determinations of efficacy should be made in animals of the type for which vaccine is produced. If no serological test to determine immunity is available, maintenance of sufficient numbers of animals for protection tests would be a difficult task. If a serological test had been standardized to indicate immunity, however, then only serums from vaccinated animals would be required and efficacy determinations would become a simple laboratory procedure.

The utility of serological tests in determining the quality of commercial vaccines has been convincingly demonstrated with the test for distemper immunity in dogs. A serological study of puppies from immune mothers led to the development of a nomograph for predicting the age at which the litter from a bitch with known titer would lose maternally transferred antibody and hence respond to vaccination (12). After being field tested the nomograph was put into use in an extensive distemper evaluation study conducted by the Cornell Research Laboratory for Diseases of Dogs. Concurrently, and using identical serological technics, the New York State Diagnostic
Laboratory at Cornell under the direction of Dr. T. Benson offered the nomograph service to veterinarians. All dogs were vaccinated in the field, and the only discernible difference between these two operations was in the vaccine used; cooperating kennels in the distemper evaluation study used an egg vaccine prepared at Cornell which satisfied the rigorous acceptance standards described previously, while the dogs tested by the Diagnostic Laboratory were vaccinated with commercial products. In terms of immunity defined by a titer in excess of 1:100, the results of these two investigations were widely divergent (Table 1). The 60 percent efficacy reported by Benson represents an average efficacy of a number of different commercial vaccines used by veterinarians. If some of these vaccines are as efficacious as the Cornell vaccine—and there is no reason why they couldn't be—then others must be extremely ineffective in order to bring the average efficacy down to 60 percent.

**TABLE I**

*Comparison by Serological Test of Results from the Cornell Research Laboratory for Diseases of Dogs Field Vaccination With Field Vaccination by Veterinarians*

<table>
<thead>
<tr>
<th>CRLDD Results†</th>
<th>New York State Diagnostic Laboratory Results‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Nomographs</td>
<td>No. Nomographs</td>
</tr>
<tr>
<td>93</td>
<td>686</td>
</tr>
<tr>
<td>No. Puppies</td>
<td>No. Puppies</td>
</tr>
<tr>
<td>403</td>
<td>686</td>
</tr>
<tr>
<td>No. Immune</td>
<td>No. Immune</td>
</tr>
<tr>
<td>397</td>
<td>426</td>
</tr>
<tr>
<td>% Immune</td>
<td>% Immune</td>
</tr>
<tr>
<td>98.8</td>
<td>62</td>
</tr>
</tbody>
</table>

* Period covered from January 1, 1959 to June 30, 1960.  
† Vaccinated with CRLDD vaccine.  
‡ Vaccinated with commercial vaccines.

Adoption and enforcement of procedures that regulate production of biologicals is a function of the Agricultural Research Service. In the past there were fewer products and all proceeded in good order; but, recently, increased research efforts have offered more products and other methods for evaluation whose immediate adoption might be desirable. Widespread consideration by members of the United States Livestock Sanitary Association, guided to a decision on usefulness by the Committee on Biologics and Pharmaceuticals of the United States Livestock Sanitary Association might aid both regulatory officials and producers. Certainly, it is a worthwhile aim for all concerned to seek agreement on adequate standards so there will be full confidence in immunization programs.

**SUMMARY**

Standardized procedures for assurance that vaccines will immunize are an urgent need today. Introduction of new products, singly and in combination, especially those of the live virus type make it imperative that we establish
SEROLOGICAL TESTS AS INDICATORS OF IMMUNITY

Efficacy ratings. Animal protection tests now in use do not permit vaccine evaluation unless larger numbers of animals are required. Such requirements are impractical. Serological tests, based upon statistical design, appear to offer solution if the serological test can be established as a reliable indicator of immunity.

Procedures for vaccine evaluation are outlined serologically and statistically to indicate possibilities. Examples from present knowledge, especially for distemper, show that possibilities are capable of practical application. The Committee on Biologics and Pharmaceuticals and Committee on Virus Review of the United States Livestock Sanitary Association are asked to give further consideration to these proposed standards for vaccine evaluation in order to strengthen confidence in immunization programs to prevent disease in animals.

REFERENCES

DEFENSE AGAINST BIOLOGICAL WARFARE ON LIVESTOCK

Dr. Frank A. Todd *

Washington, D. C.

Dr. LeRoy D. Fothergill, Fort Detrick, has stated: "The threat of biological warfare is very real. The potentialities of this threat for every community in our land must be examined in detail and with dedicated seriousness. The greatest threat may lie not in its capacity to kill people, but rather in the destruction of the economy through incapacitation of the working force and the reduction of crops and domestic animals."

In all wars of the past, military efforts have been devoted to the diminution of the enemy's food supply. This has always been an important objective in naval blockades. The grain-laden freighter has always been a prime target for the submarine. Major military campaigns have been won or lost depending upon the availability of food. Food always becomes a highly critical item during a national emergency. In wars it carries a priority rivalling that of ammunition. Anti-food biological warfare would play a decisive role in any war that was not decided with push-button speed. Biological warfare may find its greatest effectiveness when used for anti-crop and anti-animal purposes. Contrary to the case in anti-personnel biological warfare, the epiphytotic and epizootic potential of destructive diseases of animals and crops would be exploited by an enemy.

The ability to defend ourselves successfully in two world wars has been dependent upon the production capacity of our farms and factories. These facilities will have to be materially weakened or destroyed to tip the balance of victory in favor of the enemy.

Our strong, vigorous, and healthy nation is dependent upon meat, dairy, and poultry products, fruits, vegetables, and cereal crops for many essential elements. Our military forces rely upon foods of animal origin for 40 percent of their rations, which in turn constitute about 70 percent of the food value of the diet. Man's health and welfare are dependent upon a wide variety of uses of fiber crops, such as cotton and flax; also wool, leather, hair, and feathers derived from domestic animals and fowl. Animals are irreplaceable sources of certain biological products, including vaccines, serums, and glandular extracts necessary for the prolongation of human life.

VULNERABILITY OF LIVESTOCK TO BIOLOGICAL WARFARE

Of the three potential targets for a biological warfare attack—man, animals, or crops—the livestock population of the United States is the most vulnerable.

* Assistant to the Administrator for Emergency Programs, Agricultural Research Service, United States Department of Agriculture.

† Scientific Advisor to Commanding Officer, United States Biological Warfare Laboratories, Fort Detrick, Frederick, Maryland, from "The Biological Warfare Threat" presented at the 137th Meeting of the American Chemical Society, Cleveland, Ohio, April 1960.
This is due to the fact that the livestock population is the healthiest of any in the world, thereby making it highly susceptible to many of the foreign and very devastating diseases that have been successfully restrained from our countries. The herding instincts and feeding habits of animals make the introduction and spread of disease extremely easy. Within the United States the livestock marketing practices also provide an excellent means of introducing and quickly spreading animal diseases over a very wide area. These practices include the movement of animals from all parts of the country into central livestock markets. Here they may be purchased for slaughter or they may be purchased and returned to the farm for further feeding. The very complex movement in this process, plus the rapidity with which the movements are made, make the livestock population extremely vulnerable to disease spread.

Highly communicable and fatal diseases of livestock can very easily be deliberately introduced into the large stockyards of the country, the thousands of sales barns located in almost all farming communities, and into the livestock rest stations of our railroad transportation system.

Experience has shown how rapidly highly communicable diseases can spread throughout the livestock population of the United States, once they get into the marketing channels.

In the United States we have had a number of experiences dealing with the introduction of foreign diseases and pests into this country. Foreign diseases have gained entrance through the media of raw garbage, laboratory cultures, contaminated feeds and biologics, importation of breeding animals, imported plants, fruits, vegetables, packing materials, and infested materials of all types and description.

Several of the foot-and-mouth disease outbreaks that have occurred in the United States in the past have been introduced through the feeding of raw garbage from ships of foreign countries.

In 1952 a disease of swine known as vesicular exanthema, closely resembling foot-and-mouth disease, was transmitted and widely spread throughout the United States through the feeding of raw garbage. Because of the established fact that garbage can transmit certain serious animal and poultry diseases, all states now require the cooking of garbage prior to feeding it to animals. All garbage from foreign ships must either be disposed of at sea or destroyed under the supervision of a Department of Agriculture inspector.

Several unusual outbreaks of animal diseases have been related to the feeding of contaminated feeds. Unusual outbreaks of anthrax have occurred in this manner. In 1952 imported bone meal contaminated with anthrax spores was associated with unusual outbreaks of this disease in livestock. Since that time, laws have been enacted requiring all bone meal imported for animal feeds to be sterilized before its entrance into the country.

One outbreak of foot-and-mouth disease in the United States was related to a culture of smallpox virus imported from Japan to be used in the production of smallpox vaccine.

Several countries have experienced unusual outbreaks of animal diseases following the use of contaminated biologics. Hog cholera, anthrax, scrapie,
pullorum disease, and Newcastle disease are but a few examples. In these cases, a biologic used to protect against a disease was, in reality, introducing or perpetuating one.

A highly lethal disease of poultry known as fowl plague was introduced into the United States through the careless disposal of a laboratory culture following the completion of laboratory experiments. This culture was brought into the country without permission from the government. The outbreak resulted in serious losses to the poultry industry of New York and New Jersey.

**Biological Warfare Against Livestock**

There are well-known diseases and parasitic infections an enemy could use as potential weapons to attack our domestic animals as well as the human population. If our animals were left unprotected, an enemy, using these weapons, could destroy our livestock sufficiently in a few years to significantly reduce these essential agricultural resources.

In many diseases of animals, the cause and methods of transmission are well known and the measures needed for control have been established. The control and eradication of some diseases are relatively simple, of others more difficult.

The various animal diseases that might be effective biological warfare agents against the livestock and poultry of our countries include such domestic diseases as anthrax, hog cholera, and Newcastle disease. The majority of potential biological warfare diseases include those now foreign to this continent, such as foot-and-mouth disease, rinderpest, African swine fever, Rift Valley fever, and fowl plague.

Several of these diseases are widely spread throughout the world today. Several have gained entrance into the United States and Canada in the past but were quickly stamped out. Some have never appeared within our country. Our livestock and poultry are susceptible to various exotic diseases since there is an absence of immunity either naturally or artificially acquired.

There are more than 80 animal diseases and parasitic infestations transmissible from animals to man. The list of biological warfare agents usually discussed in literature as potential diseases against man includes those causing brucellosis, psittacosis, Q fever, tularemia, plague, anthrax, botulism, and typhus. Most of these maladies are common to man and animals. Epidemiological and epizootiological studies of acquired infections reveal that some of the causative organisms of this group can produce airborne infections. For many of those diseases, control or eradication of the disease in animals provides the only really logical and effective means of eliminating human infection during naturally occurring outbreaks.

Therefore, if such diseases were to be deliberately introduced and spread, there would be two potential targets—man and animals.

The deliberate and planned introduction of disease could create many problems in disease prevention and control. The animal host, disease, time, and place of introduction can be selected by the enemy. Under such circumstances, a single disease or combination of disease agents can be employed
simultaneously. Unfortunately, there are a number of animal diseases, native and foreign, benign and fatal, that present similar symptoms, all of which increase diagnostic problems. Again, even if we become acquainted with a given exotic disease, and the phenomena associated with its natural occurrence, its willful use by an enemy could involve unnatural means for dissemination, thus posing unexpected situations and compounding the problem. Several examples follow.

VESICULAR DISEASES

The first group of diseases include vesicular exanthema, vesicular stomatitis, and foot-and-mouth disease, all highly communicable. They present identical symptoms and are extremely difficult to differentiate, based on clinical observations. Laboratory or biological tests must be relied upon for specific diagnosis. Each disease possesses several types or strains of virus and within these strains are variants, further complicating diagnosis and control. Vaccine and serum effective against one type will not protect against another. Mortality in adult animals is relatively low but the presence of these diseases can result in significant losses of meat and dairy products. Abortions and high mortality among young animals, with impairment of the reproductive system, and often a general unthriftiness, result from these infections.

Foot-and-mouth disease is among the most dreaded of animal scourges. It is extremely contagious and capable of affecting any cloven-hoofed animal.

Foot-and-mouth disease is a very costly disease wherever it is present. The outbreak occurring in Mexico between 1947 and 1956 cost the United States approximately $134,571,053 to help eradicate and prevent it from entering this country. The 1952 outbreak of foot-and-mouth disease in Canada involved only 42 premises yet cost the Canadian government over $890,000. Here again the United States spent over $683,000 to protect the livestock industry of this country from this disease outbreak.

Foot-and-mouth disease has gained entrance into this country six times since 1902. These outbreaks have resulted in the loss of over 325,000 domestic animals, over 22,000 deer, and the monetary losses, both direct and indirect, were over 390 million dollars. These figures are based on the economics at the time of each outbreak (1902-1929). It is estimated that an outbreak of foot-and-mouth disease in the United States under the present marketing procedures and with current economic levels would cost this country over one-half billion dollars.

In the Mexican foot-and-mouth disease outbreak, approximately 1,000,000 animals were disposed of; 60,000,000 doses of vaccine were used; 17,000,000 animals were inoculated; and 250,000 square miles were involved. In Canada, with only 42 premises involved, 1,734 animals were destroyed.

The vesicular exanthema outbreak in 1952-1956 resulted in the loss of 1,000,000 animals, cost $22,000,000 to eradicate, and the annual cost to the livestock industry, including the biological industry, packers, breeders, sales barns, was over $20,000,000 in indirect losses.
We must be concerned seriously with each outbreak of vesicular disease in this country, whether vesicular stomatitis or vesicular exanthema is suspected, because there exists the hazard that each such outbreak may be masking foot-and-mouth disease.

**RINDERPEST**

A second group of diseases, each presenting symptoms similar to the other, includes rinderpest, mucosal disease, and virus diarrhea of calves. Rinderpest can be confused readily with virus diarrhea of calves and with the mucosal disease of cattle widely scattered throughout the dairy and beef areas of this country.

The virus diarrhea is an acute, contagious, transmissible disease of cattle. Animals can be infected with this disease in a mild form that may go unnoticed. The disease may vary from this form to a severe type closely resembling rinderpest. Animals with an extremely mild form of infection can act as carriers. Virus diarrhea is readily transmitted and can be carried from farm to farm unless preventive measures are observed.

Differentiating virus diarrhea from rinderpest is difficult. Rinderpest, in a highly susceptible animal population, has an extremely high morbidity and mortality rate. This has not been the case with virus diarrhea. Serum neutralization tests and animal inoculation tests can be used to confirm a diagnosis.

Mucosal disease is a severe disease of cattle resulting in high mortality with signs, symptoms, and pathology very similar to those of rinderpest. At times biological tests must be resorted to in order to differentiate it from rinderpest.

Rinderpest, with a mortality from 75 to 90 percent, is considered one of the most serious and devastating diseases of cattle. It greatly impairs the livestock resources of those countries where it occurs.

**SWINE FEVER**

A third group of diseases presenting similar symptoms includes hog cholera, swine erysipelas, certain nutritional deficiencies of swine, Teschen disease, and African swine fever.

Hog cholera variants have already provided many unpleasant experiences and devastating results in parts of the swine-producing areas of this country. During the years 1949 and 1950 an unusual and highly pathogenic variant of hog cholera virus suddenly appeared in the midwestern states of this country. Heavy losses were experienced on all farms upon which it appeared. The variant virus possessed low antigenic properties and thus did not produce a good hyperimmune serum or vaccine. Established dosages of protective biologics against the classical virus would not protect susceptible swine from these variants.

Teschen disease is a virulent, contagious, encephalomyelitis of swine, characterized by manifestations of a cerebral and medullary nature. It is
caused by a filterable virus. It has become a serious problem in the swine industry of several eastern European countries. In 1946, Czechoslovakia alone lost an estimated 50,000 pigs from this disease. It resembles, but is immunologically distinct from poliomyelitis. The lack of established epizootiological factors makes this disease a difficult one to control or eradicate once it makes its appearance.

African swine fever is a form of swine fever (hog cholera) of a particularly virulent character usually manifesting itself in the form of an epizootic. It has been reported by workers in East and South Africa. Recently it has been reported in Spain and Portugal. If it should spread into France and throughout Europe, the losses in the swine population of that continent could be extremely serious. Although its symptoms and pathology closely resemble American hog cholera, there are differences. Experiments in the injection of pigs immunized to hog cholera with blood from cases of African swine fever resulted in 100 percent mortality. Similar results were experienced when hyperimmune pigs were exposed. Hog cholera antiserum of either European or American origin provided no protection against the virus of this disease. To date no protective biologic has been successfully developed against African swine fever. Work on this problem is currently being carried out in East Africa.

Warthogs infected experimentally with the virus of East African swine fever did not develop symptoms but continued to harbor the virus in their blood. Normal appearing warthogs and wild hogs from infected areas harbored the virus of this disease.

RIFT VALLEY FEVER

A fourth group of diseases of this type includes enterotoxemia, vibriosis of sheep, bluetongue, Q fever, and Rift Valley fever.

Rift Valley fever is an important viral disease of man as well as domestic animals. Because man and animals are equally susceptible to it, it presents a public health problem similar to Q fever. Although Rift Valley fever is transmitted by mosquitoes, it is now clear that it can be spread by direct contact.

Rift Valley fever produces an influenza-like infection in man. Reports state that veterinarians who have conducted post-mortem examinations on animals infected with this disease have become ill. The same applies to farmers who have attended infected herds and flocks. Butchers, delivery boys, and housewives who handled infected meat that had been inspected for human consumption became ill. Cows artificially infected produced infected milk; during this period no symptoms were present in these cows except a transient fever and slight drop in milk production. Rift Valley fever has a rapid course, is highly fatal in lambs, pregnant ewes and cows, and is associated with high abortion rates. The virus is not killed by freezing but is destroyed by pasteurization. In some human cases there are serious eye complications, such as detachment of the retina, but ordinarily the course is approximately
four days. High morbidity and mortality rates, especially in lambs, high abortion rates in ewes and cows particularly, and an influenza-like disease occurring in those persons who have been associated with infected animals or material, are suggestive of Rift Valley fever. A definite diagnosis can be made by serological means, serum neutralization, and particularly, complement-fixation tests.

Rift Valley fever has been complicated by the finding of a similar condition known as Wesselsbron disease. The causal agent is antigenically not related to the virus of Rift Valley fever. It is, however, very difficult to differentiate between the two ailments on the basis of epidemiology, clinical syndrome, and pathology.

FOWL PLAGUE

Fowl plague is a fatal disease in chickens and turkeys. It occurs in Europe but may be present in Asia and North Africa and possibly South America. The disease appears simultaneously throughout an infected flock, producing death in two to four days.

There are other foreign diseases of livestock which could cause large losses in the various domestic animals if successfully introduced into this country. Information and experimental data reveal that some are difficult to transmit experimentally, being dependent upon an exact environment in which to multiply and spread. This line of reasoning has been presented relative to contagious bovine pleuropneumonia. However, man is capable of altering the characteristics of a microorganism by selecting variants with increased resistance and pathogenicity.

We are apt to emphasize foreign animal diseases when we speak of biological warfare against animals. We must not, however, forget for a moment those diseases already present within our country as current and potential disease problems. Some of these diseases could cause as much damage as any foreign plague if allowed to run unchecked among our farm animals.

ANTHRAX

Anthrax has been a problem and has been the subject of lengthy and deliberate study in many parts of the world. It is recognized as one of the oldest and most destructive diseases of animals recorded in history. From an economic and public health standpoint, it is still one of the most serious problems with which animal health officials have to deal.

Sporadic cases of anthrax have appeared in isolated areas in many states. An unusual outbreak appeared in several midwestern states during the winter and spring of 1952. These outbreaks were unusual in that (1) swine were infected almost entirely with only an occasional case reported in cattle; (2) the disease appeared during a season of the year when naturally occurring outbreaks are usually not reported; and (3) the areas involved are not considered enzootic anthrax areas. Epizootiological investigations revealed that the common factor present was imported bone meal used in feed concentrates for pigs, especially brood sows.
Protective Measures

Defense against biological warfare on agriculture begins at our borders. The Department of Agriculture maintains specially trained inspectors at border, sea, and airports to prevent the accidental introduction of plant and animal diseases, harmful insects, and other pests from abroad. In the United States this work is conducted in cooperation with the United States Public Health Service and the Bureau of Customs. This protective service has been successful; however, it must be recognized that the deliberate introduction of diseases and other pests is very difficult to detect. Border inspections and quarantine, however, do reduce the opportunities for purposeful introductions and the service has been especially alerted for this threat.

The second line of defense is formed by an inspection force of highly trained professionals ready to combat diseases and pests of both foreign and domestic origin.

Basically, our defense against biological warfare on animals is a good, sound, effective organization for communicable disease control. We are fortunate to already have established well organized and experienced cooperating disease control agencies which are charged with this responsibility.

The Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, works on the national level and deals with the problems relating to interstate disease control programs. It cooperates with the State livestock sanitary authorities concerned with the disease problems within each of the states.

The normal peacetime organization of the Department of Agriculture for animal disease control includes 49 field headquarters, usually located in the state capitals. The Animal Disease Eradication Division is in direct contact with and operates under cooperative agreements with the state livestock sanitary officials. The state livestock sanitary official, generally the state veterinarian, conducts the animal disease control program within the state by means of his own organization and with the collaboration of the United States Department of Agriculture personnel and veterinary practitioners of the state. This combination of federal and state organization for disease control extends into practically every livestock community in the country.

EMERGENCY DISEASE ERADICATION ORGANIZATION

Within almost all states an Emergency Disease Eradication Organization has been established and is on a standby basis. It provides a uniform approach to disease eradication by each state. The organization is composed of both state and federal personnel. It is based on experience acquired in the past in dealing with highly communicable, fatal, and dangerous foreign animal diseases.

The plan is one of action. Individuals are selected in advance to serve in key positions, should an animal disease emergency develop in any state. The key man is one appointed to head the emergency disease operation, under the supervision of the state veterinarian and the federal veterinarian
in charge. The organizational staff consists of an information officer, administrative officer, supplies and maintenance officer, and an operations officer. This group develops the methods of procedure. Field units that conduct the actual operations function under this staff.

The program for control and eradication is based on the following:

1. Report any suspicious condition resembling an exotic disease promptly to your state or federal regulatory official.
2. State or federal regulatory official will place quarantine on premises until diagnosis is made.
3. Veterinary inspection of adjoining premises will be made to determine extent of infection.
4. All shipments of livestock to and from the premises will be checked for the source of the diseases, as well as the possibilities of spread.
5. Test material will be sent to designated laboratories for diagnosis, if necessary.
6. Once diagnosis is made, if it is an exotic disease the veterinarian in charge will place into effect emergency operations.
7. Emergency operations will consist of quarantine; inspection and diagnosis; slaughter, if necessary; vaccination, if appropriate; cleaning and disinfection; and other measures depending on the condition involved.

**STOCKYARD INSPECTION**

One of the important defense measures against biological warfare on livestock is found in our public stockyards. Here livestock and veterinary inspectors who are trained and experienced in the detection of foot-and-mouth disease and other foreign animal diseases are constantly on the watch for animals showing symptoms suspicious of these diseases.

As far as possible, all livestock entering these markets are inspected at the time of unloading or before they are sold. If signs of any communicable disease are detected, all infected and exposed animals are promptly segregated and treated or otherwise handled in accordance with United States Department of Agriculture regulations. The state veterinarian of the state in which the diseased shipment originated is immediately notified.

**MEAT INSPECTION SERVICE**

The Federal Meat Inspection Service of the United States Department of Agriculture is set up to assure the American consumer that the meat and meat food products destined for interstate sale are safe, wholesome, unadulterated, and not mislabeled.

To aid the inspector in his work, there are seven field meat inspection laboratories located strategically throughout the country. These laboratories test and analyze specimens and samples of meat and the materials used in connection with their preparation.
About 100 million animals are examined each year by the Federal Meat Inspection Service. This represents about 80 percent of the commercially slaughtered meat animals in the United States.

Because of the large number of animals inspected and because of the ante- and post-mortem inspection procedures, the Meat Inspection Division of the United States Department of Agriculture can aid in early recognition of the presence of unusual or foreign animal diseases that might be deliberately introduced into the country.

When unusual diseases are observed by the meat inspectors, immediate notification is made to those officials responsible for animal disease control and eradication.

**VETERINARY PRACTITIONER'S RESPONSIBILITY**

The local practitioner with his experience and knowledge of the usual diseases and the normal incidence of disease in his community will probably be the first person to realize that there is an unusual increase in the incidence of any disease or that a new type of disease is present. When unusual diseases or conditions are detected by the practitioner, he reports his suspicions to the state veterinarian immediately. In the case of vesicular diseases, such as foot-and-mouth disease, and other suspected foreign animal diseases, there are specially trained diagnosticians of the United States Department of Agriculture strategically located throughout the country who can be called to conduct the necessary investigations to establish the diagnosis and instigate the necessary quarantines and eradication or control measures.

**LIVESTOCK OWNER'S RESPONSIBILITY**

The livestock owner can be extremely helpful by immediately reporting unusual conditions among his livestock to the local veterinarian. The effectiveness of a program of this type is dependent upon the efficiency and cooperation of the practicing veterinarian. It is he who maintains the preventive program of immunization and provides information through education.

**DIAGNOSTIC SERVICES**

Diagnostic laboratories are available in each state. The Department's new research laboratory at Ames, Iowa, could be used in helping to establish the diagnosis during an emergency. The large foreign animal disease laboratory of the Department which is located at Plum Island would play an important part in problems dealing with foreign animal diseases.

**INFORMATION AND TRAINING PROGRAMS**

The Agricultural Research Service, in cooperation with the United States Livestock Sanitary Association, has prepared and distributed information on biological warfare as it would affect the crops and livestock of this country. This includes information on specific animal and crop diseases and insect
pests that are looked upon as potential biological warfare threats. Short courses have been conducted throughout the country for professional and scientific personnel on identification, means of prevention, control, and eradication. Visual aids in the form of color, sound movies and slides have been developed showing in detail the means of diagnosis, identification, effects, and control and eradication procedures. These training aids are used not only by the regulatory officials but by the professional and scientific groups within the states and by the colleges and universities for instructional purposes. The movies have been used, and can be used, on television to provide widespread information on particular disease and insect problems and control programs. Of course, radio is used as required to provide the general population with information in this field.

RESEARCH

Research is conducted by the Department not only in its laboratories in the United States but in many foreign countries throughout the world. The foreign animal disease laboratory at Plum Island is working on problems of foot-and-mouth disease and other selected foreign animal diseases. In this laboratory, techniques are being developed on improving the diagnostic procedures for foreign animal diseases. American research scientists are present in several laboratories in Europe and Africa.

SUMMARY

The importance of food and other agricultural resources required during a national emergency have been discussed. The presentation includes information on the vulnerability of the livestock of this country to a biological warfare attack. Several examples have been given of experiences with past outbreaks of serious foreign animal diseases and the losses that have resulted. The defense and protective measures now conducted at the borders and all ports of entry are discussed. The State-Federal Emergency Animal Disease Eradication Organization and operational procedures are briefly summarized.
REPORT OF THE COMMITTEE ON BIOLOGICS AND PHARMACEUTICALS


Your Committee has reviewed developments this past year pertaining to biological and pharmaceutical products in order to focus your attention on those developments which appear to be the most significant. In this review we considered not only products which were new but also a great deal of emphasis has been placed this year on the re-evaluation of products which have been available for some time.

BIOLOGICALS

In the biological field, the Animal Inspection and Quarantine Division of the Agricultural Research Service, with industry cooperation, has been actively involved in the re-evaluation of rabies vaccine, modified live virus, chick embryo origin. It was learned, in the Fall of 1959, as a result of testing vaccines from several producers, which had been purchased on the market, that many of the samples tested did not show adequate potency. Therefore, a program was initiated on November 5, 1959, by the Animal Inspection and Quarantine Division to shed more light on the potency stability of this product as produced by each producer. Another regulatory memorandum was issued in April of 1960, pertaining to the testing of this vaccine and the intervals after production that tests would be required to further establish the stability. This memorandum specifically required additional testing according to a standard procedure. Licensed manufacturers, in addition to the testing of fresh material for original release, are required to retest each serial at five to seven months of age and again at 12 months of age. Samples of vaccine for such testing must be those returned from the manufacturer's supply houses. The Division also started spot-check testing of the vaccine at its Michigan State Laboratories.

The purpose of this testing program is primarily to establish the stability of vaccine produced by the different licensed establishments. The latest information available indicates that the Division has completed tests on 60 serials of vaccine representing the products of each licensed manufacturer, with the exception of one; that producer has discontinued production. These tests for the 60 serials of vaccine, along with the results of total testing by each manufacturer indicate that not all production lots pass the required tests.
for potency; or, specifically the results have indicated three general divisions of vaccine stability by manufacturers as follows:

1. Vaccines which generally do not pass the test at five to seven months.
2. Vaccines which generally do pass the required test at the five to seven month period but not at the 12 month period.
3. Vaccines which generally pass the potency tests at about the 12 month period.

Licensed manufacturers, based on the above data, have been notified to revise their expiration dates to bring them into line with the data on stability of their product as follows:

1. No expiration date
2. Seven months from the date of harvest
3. Twelve months from the date of harvest

The Division intends to continue the testing of rabies vaccine, modified live virus, chick embryo origin, and licensed manufacturers will be required to do likewise. The expiration date applied to the products of any given manufacturer could change if justified by additional data indicating longer stability than they are now able to justify.

This stability problem encountered with rabies vaccine should not be considered as a problem inherent in all desiccated live virus vaccines. There can be many factors involved in stability of living vaccines and some agents are more resistant than others. However, it should serve to focus the attention of all producers of lyophilized living vaccines on the stability of each one of them to be sure that sufficient virus or bacteria remain viable to retain their immunizing ability for the labeled expiration date.

NEW BIOLOGICAL PRODUCTS

During the past year the following biological products have been licensed:

Clostridium chauvei-septicum novyi bacterin
Anti-canine distemper and Anti-infectious canine hepatitis and Anti-leptospira canicola serum (concentrate)
Fowl-pox vaccine, liquid form
Canine-distemper vaccine, LVM, tissue culture origin
Canine-distemper virus, LVM, chick-tissue culture origin
Infectious bovine rhinotracheitis vaccine, LVM and Leptospira pomona bacterin
Mink enteritis vaccine, killed virus, mink tissue origin
Mink enteritis vaccine, killed virus, mink tissue origin and Clostridium botulinum Type C toxoid
Newcastle disease vaccine, live virus, B1 type, and Infectious bronchitis vaccine and Fowl-laryngotracheitis vaccine

This list of products illustrates the trend toward the combined immunization of animals against more than one disease, where the nature of the vaccine permits and where the combined immunization can be practically applied.
In addition to these regular licenses a special license has recently been issued for the preparation of vibrio fetus bacterin. This license will terminate September 1, 1961. In the meantime, the Division has asked the licensee to accumulate additional laboratory and field data on the efficacy of the product.

OTHER PROBLEMS IN EVALUATION OF BIOLOGICS FOR SMALL ANIMALS

An intensive program has been underway at the Veterinary Virus Institute at Cornell University, under the direction of Dr. James A. Baker, in the more critical evaluation of canine distemper vaccines primarily, but including other virus vaccines. His report, co-authored by Dr. D. S. Robson, you have already heard on this program.

He has submitted the following material pertaining to this subject for inclusion in the report of this Committee and your Committee agrees with this approach to evaluation of distemper vaccines and other virus vaccines, when and if sufficient data are available to apply them.

Biologics presently licensed and available for the immunization and protection of dogs are shown in Table I. The licensing of these products is under the control of the Animal Inspection and Quarantine Division, Agricultural Research Service, United States Department of Agriculture; control is effected by setting standard requirements in the form of specific procedures for testing sterility, stability, safety and potency, which the producer must satisfy to obtain a license and to release product for distribution.

TABLE I

Biologics Available for Protection of Dogs

Distemper
1. Inactivated virus vaccine
2. Modified live virus of ferret origin, of egg origin and of tissue culture origin (either dog kidney or chick embryo cells)
3. Antiserum

Infectious Canine Hepatitis
1. Inactivated virus vaccine
2. Modified live virus of tissue culture origin, either dog kidney or swine kidney cells
3. Antiserum

Rabies
1. Inactivated virus vaccine
2. Modified live virus of egg origin

Leptospirosis
1. Bacterin
2. Antiserum

The standard requirements for all of these dog products except the leptospirosis bacterin have been critically examined and, especially in light of
recent evidence for the existence of some low quality commercial distemper vaccine (1), it is felt that certain aspects of the standard requirements pertaining to potency, or efficacy testing should be revised and strengthened as shown in Figure 1.

After a live virus vaccine has been demonstrated safe at the lowest passage level to be used in the commercial product, the minimal immunizing dose must be determined at the highest passage level to be used; and the commercial dose, representing a suitable multiple of the minimal immunizing dose, must then be tested in susceptible dogs for efficacy at the highest passage level. In order to satisfy the recommended efficacy standards shown in Figure 1, this test should be conducted on at least 45 susceptible dogs, with additional dogs required according to the rules of a sequential test (2, 3) if any of the first 45 vaccinations fail to immunize.

Once a vaccine has passed the recommended standard requirements, continued quality control should be maintained by requiring a standardized laboratory assay of the amount of virus in each new batch of vaccine to
maintain an acceptable virus dose in the product, and by requiring annual repetition of the efficacy test in dogs. When the highest passage level is exceeded, the licensee should be required to withhold the product from the market until the higher passage level has been demonstrated effective according to the recommended standard requirements.

The prevaccination check of susceptibility and the postvaccination check of immunity in the efficacy test should be made by a standardized serological test that has been proven at least 95 percent accurate as an indicator of immunity (1). For this purpose, it is recommended that the Committee on Viruses of the United States Livestock Sanitary Association review and revise the serological tests for distemper and infectious canine hepatitis (4) and encourage the development of an urgently needed serological test for rabies.

Antiserum for distemper, for infectious canine hepatitis, for leptospirosis or combinations thereof should be standardized for antibody content by standardized serological procedures. Product for distribution should be labelled to show the number of antibody units per ml.

REFERENCES


This problem of small animal antiserum standardization is recognized by the Veterinary Biological Licensees Association.

A committee of that organization has been appointed to study this problem and come up with recommendations for standard procedures to accomplish standardization of antibody content of canine distemper antiserum.

PHARMACEUTICAL PRODUCTS

There is at present considerable confusion with regard to the Food Additive Law and the Delaney Clause and its interpretations by F.D.A. This has had a far-reaching effect on the industry producing drug products. It also concerns the veterinary profession using these products, the livestock men whose animals they are used on and also the benefactor of all this action (the consumers) of the animals or their food products. The trend in recent years to employ drug products on a mass use basis for disease prevention and increased livestock production efficiency has been definitely slowed by this legislation. In fact, it has slowed the introduction of all new drugs. It
has also caused considerable confusion with regard to the acceptability of
many preparations which have been used for a matter of years and have now
come under restriction and further scrutiny since some of them may produce
residue in milk, eggs or meat of animals or poultry.

The most recent action by Food and Drug Administration on this problem
was regarding the labeling of drugs for use in milk producing animals. This
statement published in the Federal Register, August 31, 1960, extended to all
drugs an earlier proposal to prohibit the use of injectable antibiotics and
antibiotic containing drugs in milk producing animals with a withdrawal
period of more than 96 hours. Therefore, within 90 days from August 31,
1960, the labeling of any drug which is used to treat milk producing animals
must indicate the length of time it must be withheld from human consumption
after treatment and that period must be no longer than 96 hours after treat-
ment. If treatment of these animals results in residue in the milk beyond 96
hours it cannot be used for treatment of lactating animals and must be so
labeled. Admittedly, this is, at this time, of more concern to the producers
of these drug products than to others who may use the product or own the
animals treated. However, since the object is to prevent residue in milk, it
is not unlikely that action will, in time, be taken to determine possible drug
adulteration of milk such as has already occurred in checking milk for anti-
biotic residues and accepting it only if free.

SYSTEMIC INSECTICIDES

One of the problems in livestock loss prevention which I'm sure is of
interest to this group is the progress which has been made in the evaluation
of compounds which may have value as systemic parasiticides. During the
past 10 years numerous compounds have been studied by industrial, federal
and state researchers as potential systemically-active parasiticides. Two of
these compounds have met the criteria of effectiveness and safety and are
presently available for general use against the cattle grubs. These are ronnel
and Bayer 21/199. Several promising compounds are in the final stages
of study and should reach commercial production within the next two years.

Continued research will develop simpler methods of application and in-
crease the use of such materials. Illustrative of this point is the use of high
concentrations in low volume simply poured down the back, eliminating the
need for power spray machines, syringes, or other special equipment. Such
a method also eliminates the wetting of animals in colder climates and the
need of heavy chutes for restraint.

Many of these materials show some activity against internal parasites, but
none have shown better activity than the older, established compounds. There
is hope that some materials may eventually be substituted for these older
treatments.

Emphasis in research during the coming years must be placed on the
development of chemicals and/or methods that do not leave undesirable
substances in tissues and that are not excreted in milk, regardless of the
therapeutic indication for use.
ADVERTISING OF BIOLOGICAL AND PHARMACEUTICAL PRODUCTS

This past year there has been some concern expressed in professional groups and more recently by the Federal Trade Commission pertaining to advertising of some of these agents. The recent Federal Trade Commission concern is for those products which, when used in the treatment of lactating animals, might leave residues in the milk. In line with recent Federal Drug Administration policy, only those drugs which are completely eliminated from the milk within 96 hours of the last treatment can be advertised for the treatment of lactating animals. They also want the user informed, in any advertising, just what period the milk must be withheld from human consumption after treatment and that period must reflect the research data on residues for a given product. The objective is to include in the advertising those important features, particularly the cautionary statements, regarding the use of the product which are normally carried in the direction sheet leaflet accompanying the drug.

The American Veterinary Medical Association, Council on Biological and Therapeutical Agents, has also been concerned about the advertising of all veterinary products and has setup the following criteria for advertising copy for any of the American Veterinary Medical Association, publications.

1. Advertising copy for a new product will not be considered by the American Veterinary Medical Association publications until a license has been obtained from the Agricultural Research Service (for a biological), or until a new drug application (for drugs) has become effective. Advertising copy for products or devices not regulated by the Government will be accepted at the discretion of the Council.

2. The product and the advertiser must be identified. For drugs, the full generic or chemical name of each active ingredient must be shown in six-point, or larger type. The generic, chemical or true name must be shown in connection with, or adjacent to, the trade-name of the product.

3. Advertisements shall not be misleading or deceptive.

4. Advertising copy containing statements or inferences that the advertiser is unable to substantiate will be rejected.

5. Samples or copies of the label and package insert (when used) must accompany new advertising copy for the product.

All advertisers in the American Veterinary Medical Association publications have been contacted regarding the new program and cooperation requested with respect to the above criteria. These criteria will, undoubtedly, need to be extended and perhaps modified with experience gained from their application and interpretation.

This is by no means a complete resume of developments in the field of biologicals and pharmaceuticals but your Committee members felt that these highlights might be of interest to this group.
A REVIEW OF STUDIES ON THE BIOLOGICAL NATURE OF *ANAPLASMA MARGINALE*

T. O. Roby, D.V.M., M.S.*

The biological nature of *Anaplasma marginale* has been the subject of numerous investigations during the past half-century. Yet there are still many gaps in our knowledge of this infectious agent. Differing views on the nature of the agent have resulted primarily from technical problems inherent to its study, and also from divergence in interpretation. The original protozoan concept, as proposed by Theiler (36) has persisted in the minds of many, whereas the virus-like concept, as first suggested by Seiber (31), a co-worker of Theiler and du Toit (9), has also persisted. Today, there are new research tools available to assist in studying the anaplasma (30, 32). With their aid, sound scientific inquiry and constructive debate will undoubtedly lead to a more complete understanding of the nature of this agent.

This review begins with the historical recognition of the disease, anaplasmosis, and summarizes the interpretations of successive research workers in their efforts to clarify the true nature of *A. marginale*.

**Discovery of *A. Marginale***.—It is now recognized that both anaplasmosis and piroplasmosis were frequently encountered by Smith and Kilborne (33) (34), in their studies on Texas Fever in North America. These workers made frequent references to the blue staining marginal points or coccus-like small forms situated peripherally in the erythrocytes of cattle infected with redwater or piroplasmosis. For a long time Smith and Kilborne, and also Theiler in South Africa, considered these forms to be stages in the life cycle of the piroplasma. Yet both groups of workers raised the question as to whether these forms were actually stages of the Texas Fever parasite, or of another parasite transmitted with it.

The individuality of anaplasma was definitely established by Theiler at the Veterinary Bacteriology Laboratory, Transvaal Department of Agriculture, South Africa. His historically famous report, published in 1910 covered studies during the year 1908-09. The report was titled “Anaplasma Marginale (Gen. and spec. nov.) The Marginal Points in the Blood of Cattle Suffering from a Specific Disease.” The difference between the etiology of anaplasmosis and piroplasmosis became evident to Theiler when 10 heifers previously exposed in England to South African redwater were given a second exposure to redwater upon arrival in South Africa. This was done to challenge their immunity to redwater before releasing them into the African environment. All 10 heifers reacted clinically, and five died with a disease in which the blood changes and lesions resembled redwater; however, none of the 10

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heifers developed the typical hemoglobinuria. This disease was also characterized by the "peculiar chromatic bodies or so-called marginal points." These marginal points appeared after the second challenge with redwater, but not after the original redwater inoculation in England. Theiler's sound deliberation about their mystery led him to conclude that the "immunity against Piroplasma bigeminum does not protect against infection with the marginal points." He also concluded that "they differ from any known blood parasite by the absence of a protoplasmatic body and consist only of chromatin substance, thus resembling to a certain extent the bacteria." Theiler continued, "they represent, in my opinion, a new genus of protozoa which I propose to call Anaplasma, and the species under consideration Anaplasma marginale."

Controversy began immediately concerning the soundness of Theiler's proposed protozoan concept of the anaplasma. Seiber, who worked in Theiler's laboratory, challenged this concept (31). He experimented with various blood fixatives and staining techniques, and reported that the anaplasma appear as coccus and dumbbell forms contained within a fine membrane. These structures were observed following hot alcoholic sublimate fixation and Giemsa staining. He reported differentiation of the parasites within the marginal points, and observed a single or double central body surrounded by a fine membrane. Seiber's interpretations differed radically from Theiler's, as he concluded that the coccus-like bodies were similar to cellular reaction products or inclusions as had been described by Prowazek (25) for certain viral diseases. Seiber was the first scientist to use the term "initial body" in designating the smaller bodies within the marginal points, and he described what he believed to be extra-cellular forms of the parasite having projections or tail-like appendages. Seiber stated "there is no doubt that these forms are of a parasitic nature" and "to classify the causal factor, called Anaplasma by Theiler, in the zoological system is impossible at present." Thus, the debate over the nature of anaplasma began about 50 years ago.

In the United States, Darlington (2) positively identified pure anaplasmosis infection in cattle for the first time in 1926. However, Giltner (16) gives credit to K. F. Meyer for making the first record of experimental evidence of the disease in this country in 1913.

**Evaluation of the Nature of A. marginale.**—Every research worker concerned with studies on anaplasmosis was keenly interested in seeking out more detailed information on the nature of the anaplasma. Some agreed with Theiler's opinion that the marginal points were probably protozoan in nature, while others subscribed to Seiber's belief that the marginal points were actually cellular reaction products, and that the causative agent was not protozoan but more closely related to a viral-like agent. In 1934 the characteristics of the disease, and the views regarding the nature of the anaplasma, were presented by du Toit at the Twelfth International Veterinary Congress (10). His comprehensive report clearly presented the pros and cons up to
that time, of the virus and the protozoan theories. In summation, du Toit states, "... if we take all the known facts about anaplasmosis into consideration we come to the conclusion that the simplest and most natural view to take is that this disease is caused by Anaplasma marginale and that this organism probably belongs to the protozoa." Interestingly, du Toit was one of the early scientists who had subscribed to the virus theory. Other reports on the nature of anaplasma have appeared (4) (6) (7) (19) (20). Efforts to confirm both the protozoan concept and the viral concept have been made, particularly in North American literature. The major technical difficulty, which has confronted anaplasmosis investigators from the beginning, is the lack of complete information on the life cycle and developmental stages of the agent during the entire infectious process in the diseased animal. The agent has not been successfully propagated in the laboratory or in animals other than certain ruminants. Proponents of the viral and protozoan concepts continue their differing views. More important than any individual's belief as to the biological classification of anaplasma is the acquisition of further sound scientific knowledge about the nature of the agent, so that more progress can be made in research studies and in field procedures to control the disease.

Current Studies on A. marginale.—Notwithstanding recent reports to the contrary (28), anaplasms do develop and increase in numbers in their host cell in a regular manner. This regularity in development is one of the outstanding characteristics of the disease, and was emphasized by Lotze (21), Miller et al. (22), and Hennings (18). As the anaplasma developmental cycle and method of reproduction is unknown, and as it appears to be devoid of cytoplasm, speculation continues that the anaplasma body is not an organism itself, but an inclusion type of body resulting from invasion of the erythrocyte by a much smaller agent. This concept was adopted by Foote (12), who reported a positive transmission of the disease with a Mandler filtrate collected from an acute case of anaplasmosis. Du Toit (10) summarized earlier filtration studies which had suggested the agent was not filterable. Dikmans (6) reported variable results from filtration studies, and concluded that the anaplasma may be either a reaction product caused by an unfilterable virus, or may be a protozoan parasite.

Adequate quantitative studies on the viability of the agent are lacking, although considerable work has been done with a variety of materials and methods (3) (23) (26). The viability of the agent must be considered in filtration studies, as inability to infect may result from the attendant procedures. It is now recognized that all filterable agents are not necessarily viruses (17). This is exemplified by the leptospira and PPLO groups (1). Ristic (29) has recently reported that a filtrate of lysed infected erythrocytes was infectious after passing the lysate through a Millipore filter having an average pore size of 0.65 microns. The filtrate was collected into heparinized whole blood from an anaplasmosis-free animal. The author stated that "fresh whole blood proved to be a suitable substrate for acceptance and preservation
of the infectivity of initial bodies” (29). Filtration experiments in the Animal Disease and Parasite Research laboratory (37) have shown that an infective agent is present in plasma of an acute case of anaplasmosis, and that the agent can pass, not only through the pores of a 0.65 micron Millipore filter, but also through a Millipore filter having an average pore size of 0.3 micron. An additional protective substrate was not used to preserve viability of the agent. At present the only interpretation given these data is that an infective stage of the agent can pass through a filter having an average pore size considerably less than one micron, and that the agent does exist extracellularly in the plasma of an acute case of the disease. It is also known that the disease can be mechanically transmitted by minute quantities of whole blood and that the infectivity titer of whole blood is in proportion to the number of parasitized cells (15).

The limited biochemical investigations to date have not been of great help in classifying the anaplasma. Moulton and Christensen (24) studied the histochemical nature of *A. marginale*. They found positive reactions for desoxyribonucleic acid, ribonucleic acid, protein and organic iron. They pointed out that “on the basis of the histochemistry of the anaplasma bodies alone, no differentiation can be made as to whether or not they represent bacteria, viruses or protozoa.”

Typical anaplasms are generally described as being round or oval, deeply staining chromatin masses, and measuring 0.2 to 0.9 microns in diameter. However, atypical structures and shapes have been reported by numerous investigators (5) (7) (11) (14) (20) (35). At times, comet, ring, and dumbbell forms, as well as tail-like projections and other protrusions, have been seen. They have appeared either in certain animals or only on certain days in a given animal. Although these forms may be associated with developmental stages, their relationship to the life cycle of the anaplasma has never been established. Observation of the anaplasma’s inner structures has resulted in two conflicting theories on its reproductive method: (1) multiplication by binary fission, resembling bacterial division, and (2) multiple fission, such as occurs asexually in some species of protozoa.

The smallest anaplasms visible with the light microscope are 0.2 to 0.3 microns in diameter. The greater resolution and magnification of the electron microscope provides means to observe in greater detail the morphological characteristics of the anaplasms. Electron microscopic studies of ultra-thin sections of infected erythrocytes have been reported by two investigators (13) (27). These authors have interpreted the electron micrographs of the sectioned anaplasms to be structures lacking typical characteristics of protozoa, and one of them (13) states that “these observations support the idea that the etiologic agent of anaplasmosis is a virus.” It is of interest that these ultra-thin sections of anaplasms have shown from one to eight smaller bodies within the larger body. This finding agrees with the report of Lotze and Yiengst (20) who also observed eight smaller bodies, using the ordinary light microscope. In evaluating and interpreting morphological forms of the anaplasma it is important to consider the effects of the method of fixation and
FIGURE 1—Diagrammatic representation of several forms of anaplasma as revealed by the electron microscope:

A—typical anaplasma in a bovine erythrocyte, Giemsa stain, as seen in a light microscope.
B—ring form (Espana—1959).
C—ultra-thin section of anaplasma (Foote—1958).
D—ultra-thin section of anaplasma (Ristic—1960).
(The relative sizes of B, C and D are not indicated in this drawing.)

preparation on the specimen. Electron microscopy offers definite advantages in magnification, but the infected erythrocyte must either be fixed, embedded, and sectioned, or, lysed and shadow-cast with a metallic substance. These procedures may alter the native structure of the anaplasma. Also, detailed electron microscopic studies on the blood of uninfected cattle are needed for comparison with similar studies on the blood of infected cattle. The evaluation and interpretation of electron micrographs of cattle blood constituents is a relatively new area of research and conclusions should be drawn cautiously.

The work of Espana et al. (11) and Summers (35) illustrates how different methods of preparation of the specimen may influence the appearance of
structures seen within the erythrocyte. They used lysing techniques to remove the hemoglobin from the infected erythrocyte, leaving the stroma and anaplasma for viewing in the phase and electron microscope. They observed ring and tail forms of the anaplasma, somewhat similar to those reported by previous workers using the usual blood films. It is not known whether the lysing of the erythrocyte alters the true configuration of the anaplasma body, or if structures not visible in the intact cell are revealed by the removal of the hemoglobin. Both approaches, ultrathin sectioning and erythrocyte lysing, have a useful place in studies on the morphology of the anaplasma. Figure 1 is a diagrammatic representation of several structural forms of the anaplasma as revealed by electron microscopy. It illustrates the similarities of the internal structures (C & D) found by two different research groups who made ultrathin sections of infected erythrocytes. The ring form of anaplasma (B) was observed by another group of workers who prepared the specimen by lysis. It is interesting to point out that one major difference between C & D is the double membrane surrounding the three small inner bodies in D.

Whether the etiological agent of anaplasmosis is more like the one-celled animal group, the protozoa, or the one-celled plant group of bacteria, rickettsia and viruses, is not possible to say at present. However, the evidence to date strongly suggests that this agent is a complex parasite. There are other microorganisms which are intermediate between animals and plants. An example is the flagellate, Euglena, which moves about like an animal; it contains green plant pigment and makes its own food. The anaplasma may be equally as difficult to place in an orderly biological classification. Only four years ago an authority on protozoan diseases wrote “the exact nature of the anaplasma is still undetermined” (8). About the same time another authority on bacterial and virus diseases wrote, “... the life history of the parasite of anaplasmosis is wholly unknown and therefore its proper classification is problematical” (17). It is apparent that much more research on the detection and characterization of all stages of the agent causing anaplasmosis must be pursued before the true nature of the agent can be ascertained with a satisfactory degree of certainty.

SUMMARY

The biological nature of the anaplasma has intrigued and challenged research workers for over fifty years. There has been a continuing debate as to whether the infectious agent responsible for anaplasmosis is related to the protozoa or to the viruses. Morphological characterization of the anaplasma body by electron microscopy has revealed in greater detail inner structures which closely compare with some forms observed by the earlier investigators, and strongly suggests that the agent causing anaplasmosis is a complex parasite. Continued studies of the agent concerning size range, morphological and biochemical properties, life cycle, and locations within the host, during all stages of the infection, are indicated.
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ANAPLASMOSIS EXPERIMENTAL FIELD TRIAL ACTIVITIES

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The activities of the United States Department of Agriculture for the control and eradication of anaplasmosis are divided in three parts: (1) basic research, (2) experimental field trials, and (3) the application of research findings to develop a control program.

(1) Basic research is carried on primarily at the National Animal Disease Laboratory at Beltsville, Maryland. Investigations are made under controlled conditions on the anaplasma parasite, its etiological nature, the method of its transmission, and its development and life cycle in cattle and vectors. The investigations include antigenic, hematologic, serologic, immunologic, bio-chemic, entomologic, electronmicroscopic, and fluorescent antibody studies, which supply most of the answers to questions arising from field trials. Hence, the close coordination between basic and field studies.

(2) The field trials are controlled research experiments under natural conditions in the field. These trials are planned to test basic information or theory developed at Beltsville and to solve specific problems under field conditions. The experiments are done under the supervision of the Animal Disease and Parasite Research and the Entomology Research Divisions in cooperation with state and federal agencies and livestock owners in the respective states.

(3) The application of research findings for the development of a disease control program is an expansion or enlargement of successful experimental field trial studies which also is a transition of research knowledge to field control officials. This portion of the work is participated in by both state and federal disease control agencies, as well as research groups that, for the most part, act as consultants for the technical guidance of disease control program development studies. Examples of such program work include the Animal Disease Eradication Division's anaplasmosis antigen production project carried out by the Texas Agricultural Experiment Station, the Hawaiian anaplasmosis eradication project, and the anaplasmosis control programs in Mississippi and Tennessee, the progress of which has been reported on at this meeting.

The purpose of this paper is to present the anaplasmosis field trial experiments now underway, the objective or purpose for which they were designed, and the general trend of the preliminary results thus far developed. Five active experimental field trials are underway. These are located at Rosedale, Virginia; Kerrville, Texas; Stoneville, Mississippi; Jeanerette, Louisiana, and Evanston, Wyoming. Each of these experimental studies has been designed to obtain specific information, thus eliminating duplication of research effort.

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The purpose of this experimental field trial is to study suspect complement-fixation (c.f.) reactions, attempt to determine if these reactions are specific or nonspecific, and try to find their cause and develop a method for differentiating between these reactions.

Our workers have found the c.f. test to be about 97 percent accurate. The three percent inaccurate results, both false positives and false negatives as identified by inoculation tests, are frequently associated with weak positive or suspect reactions. We have made continuous exhaustive studies of this problem because we expect difficulty in identifying and eliminating the final anaplasma infection from some low incidence herds. This problem is similar to the “no visible lesion” (NVL) problem in tuberculosis and to the suspect reactions in brucellosis.

This trial herd of about 3,000 cattle is located in the mountains of southwest Virginia where the incidence of natural infection is low. The herd owners are under contract and supervision to supply cattle to the Animal Disease Laboratory at Plum Island, New York, for research on foot-and-mouth disease. The herd is operated as a closed herd, and anaplasmosis c.f. tests have been made annually for the past six years.

Only one clinical case of anaplasmosis has been recognized in the herd during the six years. The infection occurred in a bull in the winter of 1957. The initial c.f. test in 1955 showed about two percent reactions (28 reactors and 40 suspects) in 3,178 cattle. The reactors were kept in the herd and slaughtered at the time of the second annual test when eight new reactors were found and also slaughtered. Ten new reactors were slaughtered after the third annual test, followed by five, three, and one reactors in each of the last three years. During the last five years, however, the suspect incidence rate has increased each year from 0.5 percent in 1956 to 1.5 percent in 1960. There were seven, eight, 15, 25, and 35 suspects, respectively, from the annual tests during this five-year period. We decided in 1956 that inoculations of splenectomized calves with blood from several of these reactors and suspects should be made to determine if we were actually dealing with anaplasmosis. Twenty splenectomized calves were inoculated from 20 of the c.f. reacting suspects or positive cattle and results showed 19 positive inoculation tests. Since that time all animals showing reactions have been held in isolation until we have completed our studies on them. During the last four years, inoculation tests with large (200-300 ml.) dosages of blood have been made from all suspect reactors into either known susceptible splenectomized calves or adult cattle at Beltsville. Only one or two infected animals have been identified by inoculation tests among these suspect reactors each year during the past four years.

During 1958, we made one additional semi-annual fall test on the main herd and obtained 40 reacting animals that, on the initial test, consisted of 15 positive and 25 suspect reactions. Inoculation tests were made and the group was retested every 30 days for five or six months. The final result...
was that most animals lost their suspect or weak positive reaction and we identified one single infected animal by our inoculation tests.

The majority of positive reactors and most of the infected animals among the suspects identified by inoculation tests have been old cows which originated from Texas and made up part of the original herd. It is known that most of these old cow reactors have been in the herd since the initial test in 1955 and it is believed that the c.f. test is failing to identify them except at certain periods in the life cycle of the causative agent. We question whether these old cow reactors are dangerous as a donor source for vector transmission in this herd. Intravenous inoculations of 300-600 ml. of their blood into susceptible animals resulted mostly in nonclinical cases with only one to 15 percent of the red blood cells affected. We recognized the potential value of the strain for premunition but serial passage of the strain resulted in virulent material which produced acute clinical cases.

The cause of the nonspecific c.f. suspect reactions in about one percent of the main herd, which occurred on each retest, is still unknown. Most of these reactions disappear in four to six weeks, and inoculation tests from them are negative except for one or two animals each year. From inoculation studies of suspect cattle, we now interpret suspect c.f. reactions in any herd test as depending upon the character of other reactions in the herd. If a herd had 30 percent positive reactors and five percent suspect reactors, we would be quite sure that most of the suspects were infected. However, if these five percent suspects occurred in a herd that was otherwise negative, we would consider them noninfected and recommend that they be retested in 30 to 60 days to determine if the reactions had disappeared. The c.f. reaction usually persists on retests of known infected suspects but the titers do fluctuate from negative to weak positive.

Kerrville, Texas (Entomology Research Station)

A herd of 38 positive-reactor cows and one positive-reactor bull, principally purebred Herefords, was purchased in 1958 from a Texas herd of several hundred c.f.-tested breeding animals. The purpose was to determine whether a clean anaplasmosis-free herd could be developed by raising calves from infected dams and isolating the test-negative calves at weaning time. If the calves remained negative, they would be used for the new clean herd.

The experiment is a cooperative study of the Animal Disease and Parasite Research and the Entomology Research Divisions. It is carried on near the Entomology Research Station for convenience in obtaining frequent and complete entomological surveys for insects and ticks. The natural disease incidence rate for the area is about 25 percent. Many deer frequently visit the pastures.

Now, two years after the infected herd was assembled, 36 of the original 39 animals are still positive, two are suspects, and one is negative to the c.f. test. Thirty calves were raised in 1958-59, of which five animals (16.6 percent) were positive reactors and three animals (10 percent) were suspects at weaning time. Of the remaining 22 negative 1958-59 calves, 11 heifers
and three bulls were placed in an adjacent pasture. These animals as short two-year-olds were still negative at the time of the last test in September 1960. Twenty-six 1959-60 calves were weaned and tested in September. The disease incidence rate was the same as for the previous year as there were four reactor, two suspects, and 20 negatives. The 20 negatives will be held in isolation and those negative to a 60-day retest will be added to the clean two-year-old herd. The 1960-61 calf crop should be dropped during November, December, and January.

This method of developing anaplasmosis-free herds is being investigated because it should be a much safer method of obtaining a disease-free herd than testing and segregation of adults, since calves under one year of age have greater natural resistance to the disease. Infectivity titration studies at Beltsville have shown that six- to nine-month-old calves may resist as much as 10 cow-infecting dosages of infectious blood. This method of testing and segregation should be particularly valuable for use in high disease incidence areas where insect populations are particularly heavy.

**Stoneville, Mississippi** (Delta Branch Experiment Station)

Cooperative federal-state experiments on anaplasmosis transmission were developed during 1959 and 1960. These studies were designed to measure the value of insecticides and antibiotics for preventing anaplasmosis transmission during seasons of heavy insect populations. The experiments are in the Mississippi Delta area where cattle deaths from the disease, not counting sickness or treatment, caused a three-million-dollar loss in 1958.

These experiments were initiated for the development of a source of potential support for c.f. testing and segregation program work in high insect population areas, as well as to try to give livestock owners something they could do to help themselves in the absence of testing when losses were heavy. Three divisions of the Agricultural Research Service have participated in the experiments in cooperation with the Mississippi State Experiment Station and Mississippi State University. The three Federal divisions are Entomology Research, Animal Disease and Parasite Research, and Animal Disease Eradication.

The insecticide requirement was for an agent which would give the animals continuous protection from biting insects. The entomologists selected one of the synergized pyrethrins in piperonyl butoxide as one of the most effective insect toxicants known. It was administered daily with an automatic electric spray as the animals passed through a gate to water. The duration of treatment has been for about 5½ months in this area.

During 1959, two herds having a similar amount of anaplasmosis infection were selected for study. One was treated and the other was used as the control herd. During 1960, two large infected herds were each divided into a treated and an untreated herd. The results of these experiments are as yet unpublished because of incomplete work, but indications are that this method of treatment has considerable merit.
Antibiotic feeding experiments are being made using daily dosage levels of tetracycline at the rate of 0.5 mg. per pound recommended by the workers at the Oklahoma State Experiment Station for preventing anaplasmosis transmission. During 1959, an equal number of reactors and negatives from clean herds were divided into two herds containing about 50 percent reactors and 50 percent negatives. One herd was fed antibiotic; the other was used as a control. For some unknown reason, no transmission occurred in either herd. The experiment is being repeated in 1960 and again there appears to be an absence of transmission in both the treated and untreated herds.

The negative transmission results in the control herds are probably related to the inadequate balance between infectivity level in the donor cows and numbers of insects necessary for disease transmission. For example, the entomologists report that the insect populations for the Delta area in 1959 and 1960 were only about 50-60 percent as heavy as they were in 1958; consequently, this could have an adverse effect on the transmission rate for anaplasmosis.

**Jeanerette, Louisiana** (State Experiment Station)

A cooperative study on an infected dairy herd is being done by the Animal Disease and Parasite Research Division, the Animal Husbandry Research Division (Dairy Cattle Research Branch), and Louisiana State University. The experiment is designed to measure the effect of anaplasmosis on milk production. Now in its third year of a proposed five-year test, the study will compare the production record of c.f.-reacting carrier and negative animals in the same herd and receiving the same rations and care. The acute disease has a marked effect on milk production. As yet, we do not have adequate statistical data to state the effect of carrier infection on milk production.

**Evanston, Wyoming**

This study concerns the duration of anaplasmosis infection of ticks in Wyoming. The Rocky Mountain wood tick, *Dermacentor andersoni*, commonly known as the Rocky Mountain spotted fever tick, has long been reported to be a vector for bovine anaplasmosis. It is easy to believe that this tick has some close connection with anaplasmosis in cattle in the Inter-Rocky Mountain states because there has been an abundance of anaplasmosis reported for most areas wherever this tick is prevalent. Another convincing argument for the tick reservoir theory is the natural environment in this high-dry inter-mountain area, which is directly opposite in most respects from the large southeastern states anaplasmosis enzootic area. In the southeast the highest incidence of anaplasmosis is almost always directly associated with water. The areas bordering the ocean, gulf, wet low lands, rivers, and their tributaries, are the high disease incidence areas. Water is always associated with high insect populations needed for mechanical transmission of the disease from animal to animal. Also, the northern boundary for this Southeastern enzootic anaplasmosis area is approximately
the "Mason-Dixon Line" extending west to the middle of Kansas. Why doesn't natural transmission of anaplasmosis occur north of this imaginary line in Iowa, Illinois, Indiana, Wisconsin, Ohio, and Pennsylvania? We believe this is due to lower temperatures and shorter summers, which unquestionably affect the density of insect populations and duration of their season. In contrast, most all of this inter-mountain anaplasmosis area is north of the northern latitude for the southeastern enzootic area. It does not sound reasonable for the same insects to transmit the disease only south of a given latitude in the southeast and north of this latitude in the west. The conclusion is that there must be a different vector.

Another discouraging factor about anaplasmosis in the western states has been the report from one of our nation's most outstanding entomologists. He has reported that the Rocky Mountain wood tick may transmit anaplasmosis infection through their eggs to their offspring, and that theoretically, they could retain infection and keep pastures contaminated for five to seven years even though cattle were kept off those pastures.

The purpose of our southwest Wyoming experimental anaplasmosis field trial is to test this pasture-tick infection theory. We searched for a suitable location to carry on the experiment and the tests are now in their second year. We wanted a high natural incidence of anaplasmosis in cattle where there were numerous *D. andersoni* ticks and a cooperative rancher with whom we could continue these studies for approximately five years.

We were fortunate in obtaining the cooperation of Dr. G. H. Good, State Veterinarian of Wyoming, and his staff, and a livestock company in Wyoming, which has operated the same ranch for more than 100 years. The Entomology Research and Animal Disease and Parasite Research Divisions have participated in this work assisted by the Animal Disease Eradication Division and the Wyoming Fish and Game Commission.

The Animal Disease Eradication Division had tested adults for anaplasmosis from this ranch herd in a 1957 survey, which indicated about a 50 percent disease incidence. We made a complete herd test in October, 1958, which showed about 75 percent positive reactions in cows, bulls, and yearlings and 14 percent positives in the weaned calves.

Two isolated pastures were selected for the proposed five-year tests. Seventy-five c.f. test negative yearling heifer calves were selected for the 1959 test animals. They were divided into one herd of 50 head from which reactors were to be removed if tick transmission occurred in order not to reinfect the ticks on that pasture. The second herd of 25 heifers was to have reactors remain in the herd to measure possible insect transmission after tick transmission had occurred. During 1959, only three heifers in each experimental pasture herd developed positive c.f. test reactions indicating a very low tick transmission rate; however, our testing methods might be criticized because we used yearlings rather than adults which were not available. During 1960, each of the two pastures were tested with 50 susceptible negatives consisting of about 25 yearlings and 25 two-year-old heifers. The tests are still incomplete, but the results to date again suggest a very low tick transmission rate.
and practically an equal number of yearling and two-year-old positive reactions have occurred. Entomologists have made frequent periodic insect and tick surveys of these herds for these two years. They report that the *D. andersoni* tick is the only tick species found in appreciable numbers from about May 1 to July 1.

One unplanned but very important entomological anaplasmosis observation has been made in connection with this work. About 900 fall-weaned calves showing 14 percent positive c.f. reactions were shipped on December 1, 1958, to an area near Stockton, California, for wintering. About 125 heifers were returned on May 15, 1959, for replacement breeding animals. Pacific Coast ticks, *Dermacentor occidentalis*, were on these animals during most of the winter season in California. The 125 yearling heifers on arrival in Wyoming still carried Pacific Coast ticks. Ninety-four percent of the animals showed positive c.f. reactions on arrival, and 100 percent had positive reactions one month later. No Pacific Coast ticks have been found to survive the Wyoming winters.

There are numerous wild animals, including deer, in the Wyoming test area and anaplasmosis infectivity studies in wildlife are being made in cooperation with the Wyoming Fish and Game Commission. However, at the present time it appears that Wyoming deer may be a less serious disease reservoir hazard than reported for California deer. Perhaps this difference is associated with the low transmission rate obtained with the Rocky Mountain wood tick in Wyoming, whereas the apparent high transmission rate of Pacific Coast ticks accounts for the deer infection in California.

As a result of these preliminary tests and observations, it is apparent that two additional things should be done to help solve this complex biological disease problem in our western states. First, there should be a study made of infected herds by testing and segregation in one or more problem areas in some different western states to verify whether or not the low transmission rate is a common characteristic of the Rocky Mountain wood tick. Second, the transmission rate of the Pacific Coast tick in California should be investigated in a manner similar to the Wyoming experiments.

The cooperation and assistance of state officials, state universities or experiment stations, and private herd owners is needed to help obtain this type of information which may result in the development of a method for the future control and/or eradication of anaplasmosis from our cattle industry.
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

M. N. RIEMENSCHNEIDER, Chairman, Oklahoma City, Oklahoma; W. E. BROCK, Stillwater, Oklahoma; T. E. FRANKLIN, College Station, Texas; R. G. GARRETT, Austin, Texas; R. I. HOSTETLER, Olympia, Washington; W. T. OGLESBY, Baton Rouge, Louisiana; T. O. ROBY, Silver Spring, Maryland; E. E. SAULMON, Washington, D. C.; F. B. WHEELER, Baton Rouge, Louisiana; E. H. WILLERS, Honolulu, Hawaii.

Anaplasmosis continues to be a major problem to the livestock industry. There has been no appreciable decrease in losses during the past year. It was brought out in committee report last year that production of anaplasmosis antigen for the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, was underway at the Texas A. and M. Experiment Station. Two million test doses of antigen were delivered in June. This was in keeping with the previous recommendation by the Committee. The cooperative agreement with the Texas A. and M. Experiment Station is being continued for another year whereby an additional two million test doses of anaplasmosis complement-fixation antigen will be produced.

Policies have been developed by the Agricultural Research Service outlining requirements and guide-lines for the use of standardized antigen. The antigen is available free of charge for research studies and cooperative control and field trials. It is recognized that the regulatory officials and the cattle industry should favor the control of the movement of anaplasmosis infected animals disclosed by the complement fixation test. The antigen is available from the Animal Disease and Parasite Research Division for research purposes and from the Animal Disease Eradication Division for diagnosis, control and field trials under memoranda of understanding between the cooperating laboratory and the respective division of the United States Department of Agriculture.

There are seventeen cooperating laboratories that have personnel trained to perform the complement-fixation test. During the immediate recent months serologists from two additional laboratories have received training at Beltsville, Maryland. The Animal Disease Eradication Division Standardized Anaplasmosis antigen is being furnished these laboratories. Serologists from additional cooperating laboratories are scheduled to receive training.

Experimental field studies are being continued in Texas, Mississippi, Tennessee, Hawaii, Oklahoma and Wyoming. The progress of field studies was brought out by a speaker at this meeting. Field trials being continued in Mississippi and Tennessee further substantiate the efficiency of the test and segregation measures for controlling and eradicating anaplasmosis from individual herds. The results obtained in herds practicing test segregation were, almost without exception, in direct proportion to the degree to which
procedures recommended in A.D.E. Memorandum No. 509.4 were followed. There appeared to be little change over last year in the vector population, particularly in Mississippi and Tennessee, and consequently in the prevalence of anaplasmosis in that area where these trials were conducted.

Dr. E. H. Willers, Livestock Sanitary Official for the State of Hawaii reports the following on their anaplasmosis program:

“The optimistic opinion expressed in last year’s report that anaplasmosis had probably been eradicated from native cattle was proven to be somewhat premature. Two native cattle and an import that had been in a herd for six years were found as reactors and proven to be carriers of anaplasmosis by calf trial. One of these native cattle had been purchased as a calf from a dairy herd that had originally shown a rather high incidence of infection. The other native animal was a herdmate of the imported animal referred to. The imported animal had given suspicious or negative reactions to the test from the beginning of the program in 1955 and did not give a positive reaction until June of 1959. Two confirming retests were made before a calf trial was started. All cattle that were in contact with these reactors will be retested until two negative herd tests have been obtained.

“Fifteen head out of 2,385 cattle imported during the year were classed as reactors and condemned. Nine of these were found on the first import test in quarantine, and six on the 60-90 day retest. In addition, six other imported animals gave 4+ reactions on initial quarantine but were proven by retesting and calf trials to have been false positive reactors.”

During the past year evidence has been found in Hawaii that indicates vaccination against leptospirosis may interfere with the complement fixation test for anaplasmosis.

Last year your Committee stated that the characteristics of the etiological agent of the disease had been studied with renewed interest revealing many interesting characteristics. A review of what is known about the etiological agent was made by a speaker at this meeting.

Much progress has been made on anaplasmosis. It has been gradual, steady progressing towards a goal of learning more about the actual disease, the agent that produces it, and the controlling of it by testing and elimination and management. Some knowledge has been gained as to the vectors and control of these, and treatment of the disease. In spite of the progress that has been made, it is the belief of your Committee that anaplasmosis becomes more and more of a problem to the cattle industry and should receive additional attention and concentrated effort. With this in mind the Committee makes the following recommendations:

1. Study of vectors concerning life cycles and insecticides should be expanded by cooperation between animal disease workers and entomologists.

2. Research should be continued to determine the exact nature of the causative agent. Without this information the production of an im-
munizing agent, the understanding of the pathology and successful treatment, is difficult.

3. The problem with the disease varies in terms of incidence, methods of transmission and reservoirs in different geographical regions of the country. Therefore the Committee recommends that there is a dire need for continued and broadened experimental studies in the various geographical areas of the country. In this way we may determine the vectors and reservoirs of infection and endeavor to determine feasible control and eradication measures.

4. Continued research to improve the complement-fixation test and further investigate a more simplified test method for anaplasmosis.

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

6. The Agricultural Research Service, United States Department of Agriculture, continue and expand their training program for serologists to make available personnel for all possible cooperating laboratories.

7. That all possible efforts be made to develop an effective inexpensive treatment.

That all possible efforts pertaining to these recommendations be carried out with the view to lessening the economic loss to the cattle industry because of this devastating, puzzling disease. That all agencies together, strive to put forth continued effort for field trial studies in the various geographical areas with these objectives in mind.
“A LIVESTOCK OWNER’S VIEWS ON BRUCELLOSIS ERADICATION”

V. C. JOHNSON, President, Dinsmore Dairy Co.,
Dinsmore, Florida

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

In presenting my thoughts on the subject suggested—“A Livestock Owner’s Views on Brucellosis Eradication”—I will in large measure be drawing from my own 50-plus years in the dairy and cattle breeding business, and it seems to me that some background would be helpful in showing you what has developed my thinking.

My first contact with “contagious abortion” was in 1907-08 in Delaware County, New York, where I was employed. The dairymen along Elk Creek were concerned about one farmer close to us who was losing a large percent of his calf crop and we were cautioned not to purchase cattle or have contact there as it seemed to be spreading to other herds and this made an impression on the young dairyman I then was.

In 1909 our family moved to Florida and the following year we purchased a small herd of mixed grade cows. Due to the Texas Fever Tick it was difficult to make herd additions but with double fencing we added purebreds of more than one breed. We made our decision for a Guernsey herd in 1918, a year after our first effort at tick eradication in 1917.

By this time our herd had grown to well over a hundred animals and we also were losing a good many calves and experienced retained placentas and loss in milk with the premature calvings. Some sanitary measures and care of the aborting cows were at first about the only means we knew to combat Bang’s disease at the time, 40-odd years ago.

In 1928 in consultation with our veterinarian, the late Dr. J. G. Fish, we drew our first blood and by splitting the blood samples, we sent the same specimens to Washington or Beltsville and to Jen-Sal laboratories.

The results were confusing, to say the least, and I presume inevitable with no well standardized antigen available to us at that time. Knowing of no effective measures then to combat brucellosis, we struggled on with what advice we were able to obtain, adding Guernseys which were probably highly susceptible from North and South Carolina in addition to some of our earlier purchases in Wisconsin. Though we tried to use care, we no doubt bought in a good deal of brucellosis together with the other clean susceptible animals. Our troubles did not diminish. About 1934, when a brucellosis program became available to us, we began testing regularly and I must say we did not know that long road ahead of us in the battle. Our herd had grown to probably over 300, but I can remember how it hurt when we drove 79 good grade and purebred Guernseys seven or eight miles to slaughter on
June 24, 1936. We received $3.90 per cwt. (3.9 cents per pound) for them and, while this was the largest number we have lost at one time, it was not the first or last blow in the fight. Reactors always seemed to bring decidedly less than other cows at the yard.

We had a "hot bug" and a heavily infected farm of 2,000 acres and to keep pace with the sale of our Guernsey milk, we were forced to keep increasing our production at the same time. We built an isolation barn in addition to the two milking barns on the farm to quarantine herd additions. In cooperation with some of the men that I call to mind—our Florida State Veterinarian, the late Dr. J. V. Knapp; Dr. T. H. Applewhite; Dr. J. G. Fish; our present State Veterinarian, Dr. C. L. Campbell; and some excellent suggestions from a great Guernsey man, the late Louis McL. Merryman—we instituted many physical changes and procedures, such as building 19 "hospital box-stalls" with concrete floors and curbs and galvanized metal sides for easy cleaning and disinfection, each stall individually drained into an outside gutter.

We also made small outside "calving lots" or "runs" that were exposed to the sunshine and were used for calving lots and then left vacant for a time.

Several miles of one-strand electric fence were built in double parallel rows six or eight feet apart to separate open and bred heifers and other sections of our growing herd. After each brucellosis test, we scrubbed and disinfected feed mangers, feeding alleys, water tanks in yards and pastures and placed tubs of disinfectants for boots and rubberware of employees in strategic positions and soaked feed sacks with disinfectant to place in some doorways and feed alleys. The federal veterinarian in charge for Florida, Doctor Applewhite, gave us the fullest cooperation and supervision and tested the entire milking herd at 30-day intervals—for a time, twice a month—and then, in an all-out effort to eradicate all possible sources of infection, we tested the herd every 10 days and still picked up a few reactors. This required almost super-human effort but finally in April, 1940, we were awarded our Certified Brucellosis-Free Certificate in our Marmarada herd which was lost 3½ years later when brucellosis again gained entrance into this herd. At about this time, in 1942 and 1943, Doctor Applewhite personally supervised the vaccination of a group of Guernsey calves from ages of four or six months to as high as 12 and 13 months and then check titers frequently over a long period of time and a very high percentage of even the 12- and 13-month old calves lost their titre and finally became clear on the blood tests. From that date until the present—17 or 18 years—we have vaccinated all calves born on the farm, both male and female, at six to eight months of age with Strain 19 and consider calfhood vaccination to be the greatest weapon of all in attaining and maintaining our brucellosis-free status in a dairy milk shed where there are only a few brucellosis-free herds.

After the break in the Marmarada herd in 1943, to which I referred, we used adult vaccination for several years and, while some may question this method and its advisability, I know that it helped us to weather the financial blow and maintain the necessary flow of milk at a critical time. In our opinion, there has been some "trial and error" in the rules and programs which have
been promulgated but from long experience, I am happy to be able to say that, for the most part, the men that I have had contact with and have worked with through many years in brucellosis and other disease control and eradication programs have been men dedicated to the task and ready, shall I say, to go "all out" to attain the goal. It is this Livestock Owner's View that a dairyman who is trying earnestly to eradicate brucellosis from a large herd with the problem of keeping up a fairly uniform flow of milk and is trying to learn all he can about the disease he is fighting needs lots of understanding and sympathetic help.

I am sure that some cattle owners have been their own worst enemies and have stood in the way of their own progress.

For the past several years we have enjoyed the status of a Tuberculosis Accredited and Certified Brucellosis-Free farm at Dinsmore, Florida, in the two all purebred Guernsey herds which we maintain separately on our 2,000-acre area and each herd has its own Free Certificate for Brucellosis and for Tuberculosis, and the herds comprise over 1,500 purebred registered Guernseys.

This, I believe, is one of the largest herd of purebred Guernseys in the world. The little book that I hold is a farm record of over 100 herd and part-herd tests for brucellosis under the state and federal cooperative programs and it only tells part of the story of the work and losses and victories. As late as 12 years ago, we found our consignments discriminated against in some of the good purebred Guernsey sales in north Georgia and the Carolinas and our cattle actually brought less money because they were calfhood vaccinated. Such has been the change in less than a decade so that now we find the discrimination in more recent years definitely against consignors of unvaccinated animals in the sales.

As I look at the maps which so graphically show the progress of the state-federal cooperative Brucellosis Eradication Program from December 31, 1954 to the present time, we can feel much to encourage us. (Note the progress by months and years; now New Hampshire is a brucellosis-free state.)

To keep a large herd clean in an infected area is a hazardous and a very difficult undertaking and I want to stress the need for area work, as I view it, rather than individual herd effort in controlling and eradicating this very costly disease. When we look back 40 or 50 years ago and the tools and knowledge available at that time, and then see the picture today with so much better understanding of brucellosis and sanitation, with the ring test and the marvelous results that have been obtained through calfhood vaccination, I should say that the picture never was as bright as it is today for the elimination of brucellosis.

However, I have attended meetings of cattlemen within the last year or two who felt that they could take an easy way out and fight the disease with half-way measures, instead of using all of the knowledge and resources at our command. Few now seem entirely indifferent but we just can't win a war with half-way measures.
When our nation is at war, we do not fight our battles only in the air—or only with infantry or with submarines—but we throw everything we have at the enemy. Yet, after the war when my wife and I visited Europe, we found many good and lovely people among the nations with whom our nation fought. However, I have never met a lovely brucellosis or tubercle bacillus. So, let us not try to fight these enemies with half-way measures . . . let us fight brucellosis and tubercle bacillus with everything we have.

We still need more educational work, it seems, and greater determination to completely stamp out this disease that we cannot successfully live with and at this point, I know, we have the proof that it can be done.
FURTHER STUDIES ON THE PERSISTENCE OF
BRUCELLA ABORTUS INFECTION IN CATTLE

G. LAMBERT, D.V.M., T. E. AMERAULT, B.S., C. A. MANTHEI, D.V.M. and
E. R. GOODE, JR., D.V.M.

The bovine brucellosis eradication program has progressed to the point
where more attention can and must be given to problem herds. It is highly
probable that development of more specific information about the nature and
course of brucellosis in cattle will aid in eliminating this disease. Early
reports on the localization and persistence of infection were limited mostly to
animals without a definite history as to date of exposure and course of the
disease. In other cases, complete bacteriological examinations were not
possible under slaughterhouse conditions.

Cotton (1) in 1913 reported that one cow had shed Brucella abortus in the
milk for 51 months.

Fitch et al. (3) culturing tissue from infected animals later demonstrated
the organism in 85 percent of the uteri that showed evidence of recent
parturition. However, they could demonstrate its presence in only 36 percent
of the uteri from infected animals not showing evidence of recent parturition.
The maximal period for which they could demonstrate Br. abortus in the
nongravid uterus following parturition was 195 days.

Doyle (2) examined tissues from 32 naturally infected cows and isolated
the organism from 13 sites in 26 cows. Brucella abortus was isolated from
the iliac lymph glands in 52 percent and from the retropharyngeal lymph
glands in 28 percent of the cows examined.

Manthei and Carter (9) demonstrated that the highest incidence of bacte-
remia occurred near the termination of the first gestation period after ex-
posure to virulent Br. abortus. Bacteremia receded rather rapidly after this
time, except in one animal, where it persisted for about two years. Genital
infection receded rapidly in most animals during the first month after par-
turition; however, it persisted for at least two years in one animal, which
failed to conceive during this time. It was not possible to demonstrate
excretion of Br. abortus from the genital tract of any of the animals between
conception and termination of the second gestation period after exposure. No
major differences were demonstrated in the course of Br. abortus infection
between artificially and naturally infected cows.

McCullough et al. (11) examined 100 brucellosis reactors at the slaught-
house and isolated the organism from 42. Their examinations disclosed the
presence of Br. abortus in most of the major lymph glands and organs.

From Bacteriological Investigations, National Animal Disease Laboratory, Animal Disease
and Parasite Research Division, Agricultural Research Service, United States Department
of Agriculture, Beltsville, Maryland.
Although considerable information has been developed to cope with the problems generally associated with bovine brucellosis, more information is needed in some phases of the disease to eliminate infection more effectively from problem herds. This report deals with sites of localization of *Br. abortus* in the body and the persistence of this infection as well as the relationship of both of these conditions to the sero-agglutinin response.

**MATERIALS AND METHODS**

The 169 cattle included in this study were animals that were infected in previous experiments (4) (5) (8) (9) (10). One hundred fifty-one cattle were artificially exposed to virulent *Br. abortus* and 18 were naturally exposed. All but nine of the 169 cattle were pregnant at the time of exposure. One hundred thirty-nine of the cattle were nonvaccinated, whereas 30 were calf-vaccinated. All of the calf-vaccinated animals were artificially exposed. The entire group of 169 cattle was subjected to serological tests before exposure and throughout the postexposure period. *Brucella abortus* was isolated either from the milk or uterine contents or both in 160 cattle at the termination of one or more pregnancies after exposure. Proof of *Br. abortus* infection in the remaining nine cattle, which were not pregnant at the time of exposure, was based upon isolations from udder secretions or blood, or upon persistent high sero-agglutinin titers, or a combination of both. None of the 169 cows was pregnant when autopsied.

Isolations of *Br. abortus* were made on one or more of the following serum potato infusion agar, modified tryptose agar or modified antibiotic medium. The tryptose agar medium was modified by deleting the thiamine hydrochloride and by adding 3.0 grams of yeast extract per liter and 7.5 percent of horse serum. The antibiotic medium first described by Kuzdas and Morse (7) was modified by replacing Albimi agar with the modified tryptose agar, and crystal violet with the less toxic ethyl violet. Circulin * was not used because production of it had been discontinued.

The procedures used at autopsy were as follows:

1. A sample of blood was collected for sero-agglutinin determination by the standard tube and plate methods.
2. The lymph glands obtained for bacteriological examination were submaxillary, parotid, retropharyngeal, mediastinal, bronchial, mesenteric, ileocecal, gastrohepatic, internal and external iliacs, deep and superficial inguinals, prescapular, prefemoral, popliteal and supramammary. In paired glands, both were examined.
3. Other tissues and fluids collected for bacteriological studies were the uterus, cervix, ovaries, kidneys, urine, liver, bile, spleen, milk, and tissue from each quarter of the udder.
4. Miscellaneous abnormalities such as enlarged joints, abscesses and hygromas were also collected for bacteriological examination.

* Upjohn.
Tissues and fluids were cultured directly on one or more of the three solid mediums previously mentioned. All inoculated plates were incubated at 37°C in an atmosphere of 10 percent CO₂ for five to 10 days, at which time they were examined for the presence of *Br. abortus*. All isolations were typed according to standard procedures (6).

For convenience in the preparation of tables, some of the isolation sites have been combined as follows: the term udder includes milk and udder tissue; urogenitals include the uterus, cervix, vagina, urine and kidneys.

**RESULTS**

*Brucella abortus* was isolated from 132 of the 169 cattle at the time of autopsy.

**TABLE I**

<table>
<thead>
<tr>
<th>Interval between Exposure and Autopsy</th>
<th>Number of Cattle Autopsied</th>
<th>Number of Cattle Positive for Br. abortus</th>
<th>Number of Isolations per Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Udder</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Supramammary Lymph Glands</td>
</tr>
<tr>
<td>3-6 mos.</td>
<td>20</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>7-12 &quot;</td>
<td>64</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>13-18 &quot;</td>
<td>11</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>19-30 &quot;</td>
<td>27</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>31-48 &quot;</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>5-11 yrs.</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>139</strong></td>
<td><strong>112</strong></td>
<td><strong>106</strong></td>
</tr>
</tbody>
</table>

Table I shows the relationship of localization to persistence of infection in nonvaccinated cattle. We failed to isolate *Br. abortus* from only 27 of the 139 nonvaccinated cattle, which became infected after exposure. There was a constant rate of infection within each group of cattle during the first four years after exposure. Localization occurred in more than one site in 112 of the 112 cattle from which *Br. abortus* was isolated at autopsy. The most frequent sites for the localization of the organism were the supramammary lymph glands and the udder, with the former having a slightly higher incidence. All but six of the 112 cows from which *Br. abortus* was isolated had localized infections of the supramammary lymph glands and udder. Two other areas where *Br. abortus* frequently localized were the lymph glands of the head and pelvic regions. The organism was isolated from either or both of these regions of 47 of the 54 nonvaccinated cattle with infections in other lymph glands. Of the eight isolations from the urogenital tract, seven were from the uterus and only one from the urine. By comparison there were only a limited number of isolations from other tissues, which included two
### TABLE II

Relationship of Sero-agglutinin Titers to *Brucella abortus* Isolations at Autopsy of Nonvaccinated Cattle

<table>
<thead>
<tr>
<th>Interval between Exposure and Autopsy</th>
<th>Negative (1:25 or less)</th>
<th>Suspect (1:50-1:100)</th>
<th>Reactor (+ 1:100 or more)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>3-6 mos.</td>
<td>2(2)</td>
<td>1</td>
<td>3(2)</td>
</tr>
<tr>
<td>7-12 &quot;</td>
<td>1</td>
<td>2(1)</td>
<td>4(3)</td>
</tr>
<tr>
<td>13-18 &quot;</td>
<td>1</td>
<td>1</td>
<td>2(2)</td>
</tr>
<tr>
<td>19-30 &quot;</td>
<td>2</td>
<td>1</td>
<td>4(2)</td>
</tr>
<tr>
<td>31-48 &quot;</td>
<td>1</td>
<td>1(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>5-11 yrs.</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>3</td>
<td>8(4)</td>
</tr>
</tbody>
</table>

( ) Denotes number of cattle from which *Brucella abortus* was isolated.
spleens, three livers, two gall bladders, two hygromas, two abscesses of the ligamentum nuchae, and one enlarged stifle joint.

The titer profiles of all of the nonvaccinated cattle autopsied at different times after exposure are presented in Table II. The highest incidence of isolations from nonvaccinated cattle was associated with titers at 200 or higher whereas approximately 50 percent of the animals with a titer of 100 were infected. Likewise, 50 percent of the cattle with suspect titers were infected, but three of the four animals were not pregnant either at the time of exposure or thereafter. Low titers are relatively common in infected cattle that have been exposed when not pregnant. The fourth animal had been infected for 48 months and had a sero-agglutinin titer that had receded from a high of 400 shortly after exposure. Her titer had been at the suspect level for more than a year. All of the isolations of Br. abortus from nonvaccinated cattle with suspect titers were from animals with titers of 50, except one that had an incomplete reaction in the 1:50 dilution and was autopsied eight months after exposure. A significant difference between the infected cattle with titers of 50 and those with titers of 100 was that isolations from the suspects were from a single site, whereas those made from four of the six minimal level reactors were from multiple sites. No differences were observed in the localization and persistence of infection between the naturally and artificially infected cattle.

**TABLE III**

Relationship of Localization to Persistence of Virulent *Brucella abortus* Infection in Calf-vaccinated Cattle

<table>
<thead>
<tr>
<th>Interval between Exposure and Autopsy</th>
<th>Number of Cattle Autopsied</th>
<th>Number of Cattle Positive for Br. abortus</th>
<th>Number of Isolations per Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Udder and/or Supramammary Lymph Glands</td>
</tr>
<tr>
<td>7-12 mos.</td>
<td>16</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>13-18 &quot;</td>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>19-30 &quot;</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>20</td>
<td>17</td>
</tr>
</tbody>
</table>

Table III presents the relationship of localization to persistence of virulent Br. abortus infection in calf-vaccinated cattle. Twenty of the 30 animals in this group were infected. Although the infection rate at autopsy was somewhat lower in these than in the nonvaccinated cattle, the general patterns of localization and persistence of Br. abortus were comparable. *Brucella abortus* was demonstrated in the udder and supramammary lymph glands of 17 cattle. Of three infected animals that did not show Br. abortus in the udder and supramammary lymph glands, the organism was isolated from only the uterus in one animal, from only the retropharyngeal lymph glands in another animal, and from both the bronchial and internal iliac lymph glands in the third
animal. Isolations of the organism were obtained from the lymph glands of the head or pelvic regions, or both, from nine of the 10 cattle that had infections of other lymph glands.

**TABLE IV**

Relationship of Sero-agglutinin Titers and *Brucella abortus* Isolations at Autopsy of Calf-vaccinated Cattle

<table>
<thead>
<tr>
<th>Interval between Exposure and Autopsy</th>
<th>Negative (1:50 or less)</th>
<th>Suspect (11:100-11:200)</th>
<th>Reactor (+ 1:200 or more)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-12 mos.</td>
<td>1</td>
<td>3</td>
<td>6(6) 2(2) 1(1) 1(1) 1(1)</td>
<td>16(11)</td>
</tr>
<tr>
<td>13-18 &quot;</td>
<td>1</td>
<td>1</td>
<td>2(1) 1(1) 4(4) 1(1) 10(7)</td>
<td></td>
</tr>
<tr>
<td>19-30 &quot;</td>
<td>1</td>
<td>1</td>
<td>1(1) 1(1)</td>
<td>4(2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3</strong></td>
<td><strong>5</strong></td>
<td><strong>9(8) 3(3) 2(2) 5(5) 2(2)</strong></td>
<td><strong>30(20)</strong></td>
</tr>
</tbody>
</table>

( ) Denotes number of cattle from which *Brucella abortus* was isolated.

Table IV shows the titer profiles of calf-vaccinated cattle autopsied at different times after exposure. The organism was obtained from 95 percent of the cattle with reactor titers (200 or higher). There were no isolations of *Br. abortus* from the vaccinated animals with titers lower than the minimal reactor level of 200. Although Brucella infection in negative and suspect calf-vaccinated animals is distinctly different from that of nonvaccinated ones, the number of calf-vaccinated animals is very limited.

Table V summarizes in detail the location of 400 isolations of *Br. abortus* from 112 nonvaccinated and 20 calf-vaccinated cattle.

Isolations were made from the following locations in order of frequency: supramammary lymph glands, udder, the iliacs, retropharyngeal, prefemoral, prescapular, bronchial, submaxillary and gastrohepatic lymph glands. There were only nine isolations from the uterus. *Brucella abortus* was never isolated from the kidneys of any of the cattle.

**DISCUSSION**

The research results reported in this paper have increased and solidified our knowledge about localization and persistence of *Br. abortus* infection in cattle, because they were acquired on animals with complete clinical, serological, and bacteriological histories before and after exposure.

*Brucella abortus* was isolated from seven of 10 cattle autopsied from 31 to 48 months after exposure. Thus, it is evident that a large majority of cattle, which become infected with *Br. abortus*, remain infected for the time they are ordinarily maintained in a herd. It is also important to emphasize that *Br. abortus* was localized in the udder and supramammary lymph glands of 93 percent of the infected animals. The significance of milk, contaminated with virulent *Br. abortus*, as a source of infection for cattle, other animal species, and humans should not be minimized. A point frequently overlooked is that *Br. abortus* is excreted in the feces of calves that receive milk from infected
FURTHER STUDIES ON BRUCELLA ABORTUS

TABLE V
Distribution of Brucella abortus Isolations Within 132 Cattle

<table>
<thead>
<tr>
<th>Regional Lymph Glands</th>
<th>Number of Cattle</th>
<th>Other Tissues and Fluids</th>
<th>Number of Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Head</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submaxillary</td>
<td>11</td>
<td>Uterus</td>
<td>9</td>
</tr>
<tr>
<td>Parotid</td>
<td>5</td>
<td>Cervix</td>
<td>2</td>
</tr>
<tr>
<td>Retropharyngeal</td>
<td>29</td>
<td>Ovaries</td>
<td>1</td>
</tr>
<tr>
<td><strong>Thoracic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal</td>
<td>2</td>
<td>Kidney</td>
<td>0</td>
</tr>
<tr>
<td>Bronchial</td>
<td>12</td>
<td>Urine</td>
<td>1</td>
</tr>
<tr>
<td><strong>Abdominal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric</td>
<td>3</td>
<td>Liver</td>
<td>3</td>
</tr>
<tr>
<td>Ileocecal</td>
<td>2</td>
<td>Bile</td>
<td>2</td>
</tr>
<tr>
<td>Gastrohepatic</td>
<td>10</td>
<td>Spleen</td>
<td>2</td>
</tr>
<tr>
<td><strong>Pelvic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal iliac</td>
<td>20</td>
<td>Gland or milk</td>
<td>109</td>
</tr>
<tr>
<td>External iliac</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inguinal</td>
<td>3</td>
<td>Joints</td>
<td>1</td>
</tr>
<tr>
<td><strong>External</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescapular</td>
<td>13</td>
<td>Hygromas</td>
<td>2</td>
</tr>
<tr>
<td>Prefemoral</td>
<td>14</td>
<td>Ligamentum nuchae</td>
<td>2</td>
</tr>
<tr>
<td>Popliteal</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supramammary</td>
<td>116</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From such excreta is a real source of infection for susceptible cattle particularly when they are pastured with these calves and their infected dams. The incidence (6 percent) of infection found in the urogenital tract of the nonpregnant cattle at autopsy was relatively low. However, this should not make us complacent since discharge of contaminated mucus from the genital tract of a relatively small number of cows in a herd can be and frequently is a serious source of infection. Past work (9) showed that repeat-breeder cows with genital infections, or cows with genital infection at termination of full term pregnancies are two of the most insidious sources for exposure to virulent Br. abortus.

Possibly, significance may be attached to the relatively high percentage of isolations of Br. abortus from the lymph glands of the head and pelvis. The high incidence of Br. abortus recoveries from the lymph glands of the head is probably associated with artificial exposure through the conjunctiva and natural exposure through the mouth. The high incidence of recoveries of the organism from the pelvic lymph glands is probably related to genital infections at previous parturitions. Since the genital tract of most cows becomes free of Br. abortus within five months after abortion, the question arises as to why some of these animals show genital infections at one or more subse-
quent parturitions. As long as *Br. abortus* can be demonstrated in the lymph
glands of the pelvis, it is reasonable to postulate that the microorganisms
can enter the uterus and reestablish infection, because of the direct functional
relationship between these lymph glands and the uterus, as well as the inter-
relationship of the lymph and blood systems.

The sero-agglutination tube test identified 128 (97 percent) of the bacte-
eriologically positive cattle as reactors and the remaining four (3 percent) as suspects. Any suspects in an infected herd, such as these four, should
never be permitted to leave the herd except for slaughter. Moreover, if
difficulty is encountered in eradicating brucellosis from a herd (problem
herd), strong consideration should be given to the removal of persistent
suspects as well as reactors for slaughter. An unexplained part of the results
was the failure to isolate *Br. abortus* from 12 percent of the cattle with reactor
titers. Possible explanations are that (1) these cattle were harboring the
microorganism in tissues other than the ones selected for culture, (2) *Br.
abortus* was present in the uncultured portion of the tissues selected, and
(3) limitations of the techniques employed.

**SUMMARY**

One hundred and sixty-nine nonpregnant cattle from which *Brucella abortus*
had been isolated at one or more previous times were autopsied and cultured
at intervals from three months to 11 years after exposure. Eighteen of these
animals had been naturally exposed and 151 artificially exposed to virulent
*Br. abortus*. In addition, 30 were calf-vaccinated and 139 were nonvaccinated
cattle.

*Brucella abortus* was isolated from 132 of the 169 animals. The organism
was demonstrated in the udder or supramammary lymph glands of 123 of
the 132 cattle. In the remaining nine animals, *Br. abortus* was isolated from
one or more tissues where localization does not occur frequently. The tissues
of cattle in which *Br. abortus* most frequently localized and persisted were
as follows: (1) supramammary lymph glands, (2) udder, (3) iliac lymph
glands, and (4) retropharyngeal lymph glands. Although isolations from
the uterus were relatively infrequent, any genital infection should be con-
sidered highly significant in the spread of the disease.

The incidence of *Br. abortus* isolations from animal tissues was relatively
constant for four years after exposure; however, there was a decrease in the
incidence of isolations from cattle after that time.

All but four of the 132 cattle from which *Br. abortus* was isolated at autopsy
had sero-agglutinin titers at or above the minimal reactor level. These four
were nonvaccinated cattle with suspect level titers. *Brucella abortus*, however,
was not isolated from 19 of the cattle with reactor titers.

Although the infection rate at autopsy was somewhat lower in calf-
vaccinated than in nonvaccinated cattle the general patterns of localization
and persistence of *Br. abortus* were comparable. Moreover, no differences
were observed in the localization and persistence of infection between
naturally and artificially infected cattle.
REFERENCES


In our report to this Association last year we emphasized the rapid advances made over the preceding five-year period in the Cooperative State-Federal Brucellosis Eradication Program. Although this favorable trend continued during the past fiscal year, progress was delayed to some extent by inadequate available funds to meet all service requirements. For the most part, the livestock industry throughout the country is continuing its cooperation in the eradication of bovine brucellosis to the extent that some areas cannot be serviced as rapidly as desired. At this stage of the eradication effort, progress is related largely to the level of financial support provided.

Each year the effectiveness of procedures employed in the program is further confirmed. This is exemplified in the progressive decline of infection rates and the relative ease with which Modified Certified Areas are being maintained. Moreover, following initial certification, most of these areas continue to show further significant reductions in the incidence of infection. As a consequence, we are moving toward the goal of final eradication far more rapidly than had been originally expected. At this time, there are no apparent obstacles to achieving this enviable position if we so desire.

At the December 1959 meeting of this Association in San Francisco, recommendations were developed and adopted for the establishment and maintenance of Certified Brucellosis-Free Areas. Shortly thereafter, they were approved by the Agricultural Research Service for use in the cooperative brucellosis eradication program. Prior to that time, no criteria were available for designating these areas. Such being the case, there was a natural tendency for complacency to develop in those areas that had achieved the modified certified status. Although these provisions are relatively new, they are stimulating renewed interest in many parts of the country for completing the eradication program.

**READJUSTMENT IN PROGRAM ACTIVITIES**

During the past year further reductions occurred in various field activities carried out in connection with the program. This was due primarily to the fact that 16 percent less state and federal funds were available to support the program in fiscal year 1960 as compared with fiscal year 1959. As a result of these financial limitations, the program had to be adjusted accordingly.

A comparison of operations conducted in fiscal years 1959 and 1960 is shown in Table I.

*Chief Staff Officer, Brucellosis Eradication, Agricultural Research Service, United States Department of Agriculture.*
STATE-FEDERAL BRUCELLOSIS ERADICATION

TABLE I
Tabulated Nationwide Report on Brucellosis Eradication Activities

<table>
<thead>
<tr>
<th>Activities</th>
<th>Fiscal Year</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1959</td>
<td>1960</td>
</tr>
<tr>
<td>Blood Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds—Lots</td>
<td>984,576</td>
<td>1,185,562</td>
</tr>
<tr>
<td>Reactor</td>
<td>81,226</td>
<td>60,835</td>
</tr>
<tr>
<td>Percent</td>
<td>8.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Cattle</td>
<td>14,168,909</td>
<td>12,468,476</td>
</tr>
<tr>
<td>Reactor</td>
<td>214,331</td>
<td>147,805</td>
</tr>
<tr>
<td>Percent</td>
<td>1.51</td>
<td>1.19</td>
</tr>
<tr>
<td>Ring Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd Tests</td>
<td>1,696,920</td>
<td>1,593,642</td>
</tr>
<tr>
<td>Suspicious</td>
<td>103,987</td>
<td>58,457</td>
</tr>
<tr>
<td>Percent</td>
<td>6.13</td>
<td>3.67</td>
</tr>
<tr>
<td>Vaccinations</td>
<td>6,702,832</td>
<td>6,438,497</td>
</tr>
<tr>
<td>County Certifications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>440</td>
<td>314</td>
</tr>
<tr>
<td>Removed</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Reinstated</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Total Certified Counties</td>
<td>1,653</td>
<td>1,968</td>
</tr>
</tbody>
</table>

( ) Actual difference.

Blood Testing: A total of 12.4 million blood serum agglutination tests for brucellosis were carried out on cattle from slightly over one million herds and lots during the period of July 1, 1959 through June 30, 1960. The 12 percent reduction in animal tests is not as great as had been expected. The 20 percent increase in herds and lots tested reflects the initial inclusion of lots represented in tests conducted on cattle moving through concentration points. During the year, both herd and cattle infection rates were further reduced to 5.1 percent and 1.1 percent, respectively. In comparison, we found 25 percent less reactor herds and 31 percent less reactor cattle than during fiscal year 1959.

Brucellosis Ring Testing: The value of the ring test is being emphasized further each year it is in operation. The prospects of attaining complete eradication of bovine brucellosis in dairy areas would be most discouraging without this test. As a complement to the blood test, ring testing has been found to be effective, practical and economical. Continued studies of the ring test have resulted in improved techniques being developed and adopted in many states. For example, it has been found that utilization of properly preserved Babcock samples for ring testing is more efficient than the original can-sampling procedure. This method is far more economical to apply and
eliminates some of the discrepancies encountered with tests run on fresh milk samples. At the present time, practically all states have adopted the Babcock sampling procedure. During the past year, approximately 30 million cattle were represented in the 1.5 million herd ring tests. This is the equivalent of one and one-half ring tests each year for all milk cows in the nation. However, this volume should be materially increased by more frequent herd tests. The fact that only 3.6 percent of the herds tested last year were considered suspicious is quite significant when compared with the 23.2 percent disclosed six years ago.

**Vaccination:** The level of official vaccinations recorded over each of the past two years has remained fairly constant at about 6.5 million. This means that we are vaccinating annually about 50 percent of our eligible calves. It is rather urgent that this level be increased to at least 75 percent if the danger of explosive brucellosis outbreaks is to be minimized. There is a tendency to consider vaccination requirements of minor importance in the Modified Certified Brucellosis Areas where, in fact, the need is still critical. Until the last infected animal has been found and removed, calf vaccination should be continued at the highest possible level.

**ESTABLISHMENT OF MODIFIED CERTIFIED BRUCELLOSIS AREAS**

The development of Modified Certified Brucellosis Areas is an effective means of advancing the eradication effort. The fact that maintenance problems become progressively less difficult as more areas become certified confirms the value of this procedure.

Because of the substantial reduction in funds available for cooperative brucellosis eradication in fiscal year 1960, readjustment of program objectives was necessary. During this time the number of new areas added to the program was decreased by more than 40 percent as compared with 1959. For the same 12-month period, initial county certifications fell about 28 percent. In most instances funds were channeled into areas where considerable progress already had been made. Also, high priority was given to the maintenance of Modified Certified Brucellosis Areas in order that their status could be retained and further progress made toward eradication.

During the 12-month period ending June 30, 1960, a total of 314 new counties qualified for the Modified Certified area designation. This compares with 440 new counties added to the list in 1959. At the beginning of fiscal year 1961, there were 1,968 certified counties, including 24 complete states, Puerto Rico and the Virgin Islands. At the same time, there were 288 other counties working directly toward certification. The annual relationship between counties certified and area-work counties for the years 1954 through 1960 is given in Figure 1.
Cooperative State-Federal Brucellosis Eradication Program

COUNTY CERTIFICATION STATUS

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Complete Area Testing</th>
<th>Modified Certified Counties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1954</td>
<td>334</td>
<td>42</td>
</tr>
<tr>
<td>1955</td>
<td>379</td>
<td>173</td>
</tr>
<tr>
<td>1956</td>
<td>500</td>
<td>335</td>
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<tr>
<td>1957</td>
<td>735</td>
<td>712</td>
</tr>
<tr>
<td>1958</td>
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<td>594</td>
</tr>
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<td>1959</td>
<td>1,653</td>
<td>492</td>
</tr>
<tr>
<td>1960</td>
<td>1,968</td>
<td>288</td>
</tr>
</tbody>
</table>

ESTABLISHMENT OF CERTIFIED BRUCELLOSIS-FREE AREAS

The ultimate goal of the cooperative brucellosis program must be eradication. In spite of strong evidence to the contrary, there are some who still believe that control of brucellosis is the most that can be expected. Unfortunately, it was not until February 1960 that procedures became available for establishing and maintaining Certified Brucellosis-Free Areas. Before that time, there was evidence of a considerable number of counties throughout the country being practically free of infection. It was not surprising, therefore, to find some of the states immediately taking an active interest in qualifying brucellosis-free areas.

On April 25, 1960, the entire State of New Hampshire met the requirements for a Certified Brucellosis-Free Area and became the first state to achieve that distinction. This designation means that as a result of tests conducted on all herds in each county over a period of 18 months, no *Brucella* infection is known to exist at the time it is declared free of brucellosis. Attention is given also to other susceptible species of domestic animals whenever there is reason to suspect their being affected.

In the case of New Hampshire, the state has supported an active brucellosis eradication program for many years. It was the second state to attain the Modified Certified Area status. Although New Hampshire is relatively small, the brucellosis problem was quite serious in 1934 when control and eradication
efforts were initiated. At that time, almost 50 percent of the herds and 10 percent of the cattle in the state were affected. In spite of this high level of infection, consistent progress was made over the years by utilizing procedures recommended by this Association and approved by the Agricultural Research Service. The culmination of these efforts resulted in the state qualifying as a Certified Brucellosis-Free Area. This accomplishment demonstrates conclusively that brucellosis eradication is a goal that can be attained by the intelligent application of available tools and knowledge. During the balance of fiscal year 1960, 12 additional counties in four other states qualified as free areas, making a total for the year of 22 free counties, including one complete state. As of October 1, 1960, the number of free areas had increased to a total of 38 counties in eight states. Figure 2 shows the extent and disposition of the Modified Certified Brucellosis Areas and the Certified Brucellosis-Free Areas throughout the country as of August 31, 1960.

FIGURE 2

MARKET CATTLE TESTING PROGRAM

The advantages of testing market cattle on the way to or at packing plants is proving to be an economical and effective method for requalifying Modified Certified Areas. While this procedure will be especially useful in range and semi-range areas it can be applied to advantage in other areas as well. In March 1960, Benton County, Washington, became the first county in the nation to be recertified by market cattle testing. In this county it was found that 92 percent of the ranch level testing, usually required for recertification, was eliminated.
Data collected beginning September 1959, show an increasing number of states having put this program into operation. Figure 3 depicts, by months, the states participating in market cattle tagging and testing. It will be noted that at the end of June 1960 there were 14 states applying back-tag identifications and 26 states conducting tests on market animals. Considering the fact that the program data covers a period of only nine months, the progress is quite encouraging. Over the nine months' period, there were 146,567 blood tests conducted on animals of this type. These tests revealed 1,093 animals—0.7 percent—with titers of 1:100 or higher. It is significant to note also that all but 71 of these reactors were traceable to herds of origin. For purposes of recertifying areas, both negative and positive test results are credited back to the areas involved. By so doing, it appears that only about three percent of the herds in beef producing areas will need to be blood tested at the ranch level. This will permit recertification of range and semi-range areas with a minimum of inconvenience to owners and at the same time provide more effective brucellosis eradication than is possible with the alternate 20 percent test method. When market cattle testing is universally adopted, the over-all savings to livestock owners will be very great indeed.

A new brucellosis color movie entitled "Traceback" has just been completed. It is designed to explain the advantages and operation of the market cattle testing program. Wide distribution of this film should help encourage
adoption of market cattle testing as a means of recertifying range and semi-range areas.

INCENTIVES FOR COMPLETE AREA WORK IMPORTANT

For the most part, the brucellosis problem has not been as serious in range as in other areas. Consequently, the need for eradication has not been as urgent and some owners have questioned the benefits to be derived from such a program. One of the most important factors involved in this regard relates to the movement of cattle from Modified Certified Areas. By providing preferential treatment for these animals, advantages can be realized by owners through the attainment of area-wide certification. While considerable progress has been made in this direction, there is still more that should be accomplished. At present, only 21 states accept cattle from Modified Certified Areas without restriction. Another 12 states are sympathetic but have not as yet, complied with the proposal. The balance of the states continue to oppose any relaxation of import requirements, regardless of the status of the areas from which cattle originate.

Beginning in July 1957, the Agricultural Research Service has undertaken to evaluate the effectiveness of modified certification procedures by testing interstate shipments originating from these and other areas. As of September 1, 1960, a total of 67,332 cattle from Modified Certified Areas and 26,309 from non-certified areas, had been tested for brucellosis at destinations. Of the 67,332 cattle originating in Modified Certified Areas, only 32 were found to be reactors. This represents one reactor out of each 2,104 animals tested. In contrast, one reactor was disclosed out of each 135 cattle tested from non-certified areas. By granting preferential treatment to cattle originating from herds not known to be infected with brucellosis in Modified Certified Areas, added inducement can be provided for early completion of the certification program on a nationwide basis. It is hard to believe that one reactor in 2,000 imports will seriously jeopardize the brucellosis status of any area. Moreover, the progress made in all areas will be increasingly protected as the certification program advances.

FUTURE PROSPECTS

State and federal funds available for the current fiscal year are 12.7 percent above the support provided last year. This situation will permit a reasonable expansion of the program in areas where the need is most urgent.

As the program moves ahead, more funds are required for the maintenance of Modified Certified Areas. For fiscal year 1961, it will be necessary to recertify about 627 counties. This compares with only 350 recertifications accomplished in fiscal year 1960.

At the present time, it appears that approximately 225 counties will be initially certified in 1961. During the first three months of this year, 69 new counties qualified as Modified Certified Areas. Because of the increased volume of maintenance work required and the shortage of field personnel in
some of the states, it is not expected that the number of area work counties will exceed 300 at any one time during the year. In fact, unless additional field assistance can be obtained in some of the 24 states not yet certified, it will be difficult to appreciably increase the present level of 291 counties working toward certification. However, if progress continues at the present rate, it is possible that all areas in the country could be on an active participation basis during the next three years, and nationwide modified certification reached by 1966.

COMMENTS

Although financial limitations in fiscal year 1960 retarded progress in many areas of the country, it was possible to qualify 314 new Modified Certified Brucellosis Areas. It should be pointed out, however, that a back-log of work already accomplished in area counties during 1959 contributed materially to the number of certifications completed in 1960.

As of August 31, 1960, 63.5 percent of all counties in the United States, Puerto Rico and the Virgin Islands were Certified Areas. By including area-work counties, 73.5 percent of all counties were either currently designated as Certified Areas or were rapidly approaching that status.

With 26 states, Puerto Rico and the Virgin Islands now Certified, it is essential that increased emphasis be placed on the establishment of Certified Brucellosis-Free Areas. The fact that 38 counties in eight different states have already achieved this goal, demonstrates the feasibility of eradicating bovine brucellosis. Thus, every effort should be made to encourage all Modified Certified states to move in this direction as rapidly as possible.

It is important that the market cattle testing program be extended in range and semi-range areas as a practical and efficient means of recertification. Through full cooperation of all concerned this procedure can be equally as important in range areas as the brucellosis ring test has been found to be in dairy sections of the country. Both of these tools are necessary if final eradication of brucellosis throughout the nations is to be achieved.

As the degree of infection reaches a low level we can expect to encounter herds in which brucellosis is difficult to eliminate. Recognizing this probability, specially trained epidemiologists are being assigned to the different states as the need becomes apparent. Supported by competent laboratory service, these veterinarians are in a position to employ an imposing array of supplementary procedures for detecting and eliminating persistent Brucella infections. During the past year, progress along these lines has been most encouraging.

So far, other reservoirs of Brucella infection have not interfered seriously with the bovine brucellosis eradication program. However, it is becoming increasingly urgent that more consideration be given to all susceptible livestock if complete eradication is to be accomplished. Although the incidence of infection in swine and goats has never been as high as originally found in cattle, it constitutes an economic and public health hazard that cannot be
ignored. With 26 states now having provisions for establishing Certified Brucellosis-Free swine herds, only 528 such herds are currently listed in 20 states. This reflects a lack of knowledge by swine owners of the advantages to be gained by eliminating infection from their herds. The educational effort necessary to correct this situation should be undertaken in all swine growing areas as part of the bovine brucellosis eradication program.

At this stage of the program, the only logical goal must be eradication. Moreover, there is increasing evidence that the attainment of this enviable status will be supported by most of those concerned with the brucellosis problem. With the additional funds available for the eradication effort in fiscal year 1961, we can look forward to significant progress being made along these lines during the next several months. The doctrine of eradication is no longer a visionary prospect, it is as realistic as the intermediate Modified Certified Area stage.
REPORT OF THE COMMITTEE ON BRUCELLOSIS


Mr. President, Members of the Association and Invited Guests: Last year in San Francisco your Committee on Brucellosis held open hearings all day Tuesday, December 15, 1959. Every individual or group of individuals who presented themselves for a hearing were given an opportunity to discuss any and all subjects relating to brucellosis eradication, and to take up such changes in the Uniform Rules and Regulations that they felt would materially assist the livestock industry and the regulatory officials in carrying out the Brucellosis Eradication Program in the United States. Wednesday, the 16th, these proposals were studied and discussed very thoroughly by members of the Committee, many of the suggestions were incorporated in the Amended Uniform Rules and Regulations, and some were rejected. This year, the procedure followed the same pattern. Tuesday of this week, October 18, your Committee was in open session all day long, and Wednesday a careful study of all proposals presented at the open hearings on Tuesday, were studied and considered. While it was the general belief of your Committee, as well as the general belief of many of the regulatory officials from many of the states of our union, that our Uniform Rules and Regulations as adopted in San Francisco provided sufficient machinery to carry on the project of brucellosis eradication for another twelve months without any drastic change, the Committee did not lose sight of the fact that consideration must be given to changed conditions, and new and important research that has been carried on during the past twelve months.

Last year Part V was added to our Uniform Rules and Regulations, which provided for the certification of Brucellosis-Free Areas. Under Part V one state, namely, New Hampshire qualified as of April 25, 1960. In qualifying for this Certified-Free Area status, the rules and regulations governing the requirements of the certification were carefully studied with a result that minor changes have been recommended in Part V of the Uniform Rules and Regulations.

Other proposed changes have been reviewed by the Committee, and some have been approved and will be presented here at this time. Your Committee is of the opinion that great progress in the program of brucellosis eradication
has been made during the past year, and this opinion is borne out by the report made by Dr. C. K. Mingle in his address to us just a few minutes ago. That there is a lot of work yet to be done cannot be denied by anyone who is in a position to know the problems that must be met in carrying out a program of this kind, but we want to emphasize here that there is no doubt in the minds of your Committee as to the final outcome.

Without further remarks, we will proceed to present to you the recommendations agreed upon by your Committee and if accepted by the Executive Committee, the General Assembly, and the Agricultural Research Service of the United States Department of Agriculture, the recommendations will become a part of the Uniform Rules and Regulations under which we will operate during the next 12 months.

The recommendations of your Committee are as follows:

1. That the Agricultural Research Service give special consideration to the removal of animals from “problem herds.” To expedite their removal by utilizing all brucella procedures that are available. Indemnity to be paid for any animal declared infected with brucellosis.

2. Recommend the Committee on Resolutions petition the Federal Congress to appropriate sufficient funds to maintain the Brucellosis Control and Eradication Program at the present level of expenditure.

3. Recommend that all states take the necessary action to have their respective legislatures appropriate sufficient monies to comply with the 60 percent Federal, 40 percent state level of program expenditures that will become effective July 1, 1962.

4. Your Committee was asked to review the requirements of the Uniform Methods and Rules that provide for the movement of feeder cattle. After due deliberation, your Committee believes the rules as currently written provide for the orderly movement of feeder cattle into either Modified Certified Brucellosis Areas or Certified Brucellosis-Free Areas.

5. The Committee wishes to extend congratulations to the State of New Hampshire for attaining the status of a Certified Brucellosis-Free Area.

6. Your Committee received the following resolution presented by Mr. Robert Laramore, Chairman of the National Brucellosis Study Committee of the American National Cattleman’s Association:

**BE IT RESOLVED THAT:**

“In any range or semi-range area any modified county or area shall be eligible to recertify by calfhood vaccination. When 80 percent of the breeding cattle in such county or area can show positive evidence to the fact that all of the heifers retained or added annually in the herd have been officially calfhood vaccinated. Any herd not so vaccinated shall be submitted to the required blood test each three years.”
BE IT FURTHER RESOLVED:

"Any herd electing to recertify under the above mentioned alternate method must have a five-year backlog of official calfhood vaccination or the entire herd has been officially calfhood vaccinated."

This resolution was given serious study and hours of deliberation. Your Committee agreed emphatically with the assertion regarding the value of calfhood vaccination. However, carefully conducted research has demonstrated repeatedly that vaccination has not and does not produce complete immunity. In fact the evidence is conclusive that approximately 70 percent protection is provided. It is obvious, of course, that safeguarding the health of the nation's livestock requires that assurance be provided that breeding animals do not constitute a health hazard to the cattle of other livestock owners.

It is the opinion of your Committee that the basic rules as written will operate satisfactorily in any area.

7. Your Committee again reviewed the Uniform Methods and Rules. There are only a few recommended changes, therefore it is suggested to the Agricultural Research Service that the proposed changes, if approved, be sent to all state and federal offices. This would enable each regulatory official to have the latest changes and would not require a reprinting of the Uniform Methods and Rules booklet.

The proposed changes are as follows:

A. Where it appears in the rules, “cull and slaughter testing” should be changed to “market cattle testing program.”

B. Part IV, Section I, Paragraph E. At the end of sentence add “in accordance with Paragraphs A, B or C of this section.”

C. Part IV, Section II, add new paragraph C (2) (f), and redesignate existing (f) to (g). New paragraph (f) to read:

“If testing accomplished under paragraph C (2) (b) is not sufficient to qualify the area for recertification, the area may be recertified by blood testing 20 percent of the herds in the area that are not represented in tests under paragraphs C (2) (b), (d), or (e). In lieu of a complete blood test, a screen test may be conducted on such herds in accordance with paragraph B of this section. The herds tested shall not include the same group previously tested for this same purpose.

D. Part V, Section I, Paragraph 2 (b), after brucellosis, insert “milk.”

E. Part V, Section II, Paragraph B 1 (a) after brucellosis, insert “milk.”

F. Part V, Section II, Paragraph B 2, after brucellosis, insert “milk.”

G. Part V, Section II, Paragraph B 1 (d), delete “within,” insert in lieu thereof, “each,” and delete, “prior to the termination.”

H. Part V, Section II, Paragraph B 5, delete this paragraph and redesignate following paragraphs as necessary.
THE RELATIVE IMPORTANCE OF VARIABLE FACTORS IN THE AGGLUTINATION-LYSIS TEST

E. A. CARBREY, V.M.D., M.S.*

Ames, Iowa

INTRODUCTION

A survey of laboratories engaged in the serological diagnosis of leptospirosis in 1959 revealed a total of 28 laboratories employing the agglutination-lysis test (1). Unfortunately, it was also found that these laboratories used 28 different methods of performing this technique. Naturally, a great deal of the variation among the findings of the laboratories was attributed to the lack of a standard protocol for performing the agglutination-lysis test. Analysis of the data from the survey indicated that some of the variation among the laboratories was due to the use of different dilution schemes, different strains of Leptospira pomona for antigen, and different tests incubation temperatures. The Committee on Leptospirosis at the last meeting of this Association recommended a standard protocol for the agglutination-lysis test which should be adopted universally (2). The following factors were considered crucial by the Committee:

a. dilution scheme
b. incubation temperature of the test
c. estimation of end points
d. strain of L. pomona
e. culture medium
f. age and density of antigen culture

With the above facts in mind it seemed logical to set up some experiments to determine just how the serological findings of the agglutination-lysis test could be influenced by controlled variation at different points in the protocol. The experiments were designed so that the variation produced by a factor under study, such as antigen strain, could be compared statistically with the natural variation or residual error of the experiment.

LITERATURE REVIEW

The agglutination-lysis test has been the universal serological technique used in the diagnosis of leptospirosis since the early work of Schuffner and Mochtar (3). Variations in the preparation of the leptosporal culture for antigen by different laboratories was noted by Borg-Petersen and Fagraeus

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FACTORS IN AGGLUTINATION-LYSIS TEST

in 1949 who had observed considerable discrepancy between tests performed on the same serums by two Swedish laboratories (4). They systematically studied the effects of antigen density and other factors on the serum titer. The methods used by other laboratories to standardize their antigen were reviewed and it was concluded that marked variations existed in the density of the antigen used by these laboratories. Precise experiments were carried out which related a twofold increase in the titer of a serum to a fourfold dilution of the antigen culture. Variation in the titer of a serum was related to the use of different strains of the same leptospiral serotype. Increasing age of the antigen culture decreased its sensitivity in the agglutination-lysis test. No effect was attributed to the use of different batches of media used to grow the antigen culture. Recommendations were made in an effort to standardize the test in regard to the factors studied and the advantages of a standard technique were pointed out in some detail.

The protocol as developed by the Dutch school was recommended by Wolff in 1954 (5). The basic features were the droplet method of dilution utilizing capillary pipets and small porcelain plates instead of test tubes. The final dilutions achieved were 1-10, 1-30, 1-100, 1-300, etc. In 1956, a study group of the World Health Organization stated that the essentials of the technique were to be found in the monograph by Wolff previously cited (6). However, this method has not been generally accepted in this country. Only one laboratory in the 1959 survey prepared their tests in porcelain plates and used the dilution scheme described. The standard protocol described in the “Report of the Committee on Leptospirosis” previously cited (2) possesses a distinct advantage over the technique described by Wolff. Most of the points defined, such as dilution scheme and culture medium, are those utilized by the majority of the laboratories presently using the agglutination-lysis test.

GENERAL MATERIALS AND METHODS

The type strain, *Leptospira pomona* Pomona, was used in all of the experiments except the experiment comparing antigen strains. In this experiment, additional strains, S-91, Clark, and Johnson were employed. The Pomona, S-91 and Clark strains were obtained from the WHO/FAO Leptospirosis Reference Laboratory, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D. C. The Johnson strain was obtained from Communicable Disease Center, Veterinary Public Health Laboratory Unit, P. O. Box 185, Chamblee, Georgia. Leptospiral strains used as antigens were maintained in Stuart’s medium (7) as prepared by Difco Laboratories, Inc. and transferred every three to five days. Approximately nine ml. of culture medium were placed in sterile screw-cap vials, 20 x 125 mm. in size. An inoculum of one ml. of an actively growing culture was added to each tube and the cultures were incubated for three to five days at 30°C. The antigen cultures were examined microscopically for growth and, if suitable, were centrifuged at 500 g on a horizontal head for 10 minutes. The antigen was removed from the culture tube and examined for density with the dark-
field microscope. Buffered saline was used to dilute the antigen when necessary. The formula for the buffered saline was as follows:

\[
\begin{align*}
\text{Na}_2\text{HPO}_4 & \quad 2.0 \text{ grams} \\
\text{KH}_2\text{PO}_4 & \quad 0.4 \text{ grams} \\
\text{NaCl} & \quad 4.0 \text{ grams} \\
\text{H}_2\text{O} \quad \text{[ion exchange]} & \quad 1.0 \text{ liter}
\end{align*}
\]

Serial dilutions of serums were made in the same buffered saline and 0.1 ml. of each dilution was placed in a small glass tube, 10 x 75 mm., with 0.1 ml. of antigen. Titers reported in each experiment were final dilution titers achieved after the addition of antigen to the serum dilution. The tubes were shaken and incubated in an air incubator for two hours at 37°C. except in the experiment on the effect of test incubation temperatures. The tests were read by placing an oblong drop on a microscopic slide from each tube with a capillary pipet and examining by dark-field microscopy at 150X magnification [Leitz Ortholux]. The degree of agglutination was read as negative, 1, 2, 3 and 4. The readings corresponded to the following degree of agglutination as judged by the number of free leptospiras present rather than the agglutinated masses.

<table>
<thead>
<tr>
<th>Degree of Agglutination</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>trace percent or less</td>
<td>N</td>
</tr>
<tr>
<td>25 percent</td>
<td>1</td>
</tr>
<tr>
<td>50 percent</td>
<td>2</td>
</tr>
<tr>
<td>75 percent</td>
<td>3</td>
</tr>
<tr>
<td>100 percent</td>
<td>4</td>
</tr>
</tbody>
</table>

The end point titer of a serum was indicated by the highest dilution showing at least 50 percent agglutination.

Further innovations were made in each experiment to study the effect of the different variable factors of the test. These changes in technique will be described in the next section.

The serums used in these experiments were from animals naturally and experimentally infected with \textit{L. pomona} and were selected for each experiment so that a wide range of antibody levels were obtained.

RESULTS AND DISCUSSION

\textbf{Replication of Antigen Lots and Dilution Sets}

This experiment was planned to determine how much variation occurred among batches of antigen culture prepared on different days and also to detect the difference between two sets of dilutions tested on the same antigen. The two factors were compared simultaneously on 16 serums. Four lots of antigen were prepared as described on four successive days. Each day two sets of dilutions were prepared so that two complete tests were performed on the same lot of antigen. The two sets were identified as "1" and "2" since there was a time lapse between the preparation of the tests from each set during which the antigen remained exposed to air contamination at room temperature. The layout of the experiment is presented in Figure 1.
FACTORS IN AGGLUTINATION-LYSIS TEST

FIGURE 1

Agglutination-lysis Test
Four antigen lots prepared on different days with two dilution sets on each antigen lot

<table>
<thead>
<tr>
<th>Sera</th>
<th>I Dilution set</th>
<th>II Dilution set</th>
<th>III Dilution set</th>
<th>IV Dilution set</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-16</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Analysis of Variance

- Error: 0.075
- Dilution sets: 0.101
- Antigen lots: 0.053

The titers of the 16 sera were transformed into logarithms and the analysis of variance was performed as described by Kendall (8). The residual variance or experimental error was found to be 0.075. The variance or mean square for antigen lots was 0.053 and the variance for dilution sets was 0.101. The degree to which these values exceeded the error variance represented the amount of variation contributed by these factors. The F test for significance was applied by dividing these values by the error variance (9). An F ratio of 0.70 was calculated for antigen lots. This value would have had to exceed an F value of 2.70 before variation among the antigen lots could have been accused of producing significant effects on the serum titers. The F ratio of dilution sets, 1.33, was considerably below the F value at the significant, or five percent level, of 3.94. Neither antigen lots nor dilution sets introduced significant variation in the testing of these serums. This is displayed graphically in Figure 1 where squares are constructed which show the relative size of these variances. The area of these squares is equivalent to the variance values which are, by nature, mean squares. This procedure of depicting the variance or mean square values as squares for visual comparison will be followed throughout this paper. The two factors examined in this experiment did not introduce significant variation; however, all of the antigen preparations and test readings were made by one worker.

Effect of Antigen Density

The work of Borg-Petersen and Fagraeus on antigen density suggested an effort to confirm their findings. It seemed reasonable that an increase in serum titer would be related to a decrease in antigen density.

A master set of fourfold dilutions was prepared using 19 serums. The final dilution titers were 25, 100, 400, 1,600, 6,400, 25,600, 102,400 and 409,600.
Four sets of tests were prepared from the master dilutions using different densities of the antigen culture. A luxuriant, four-day-old, culture was diluted 1-2, 1-4, and 1-8. The final dilution of the antigen in the four sets of tests prepared was 1-2, 1-4, 1-8 and 1-16. The tests were incubated and read in the manner previously described. The reading of the tests with the 1-2 and 1-4 antigen dilutions presented no problem. However, it must be admitted that the tests prepared with the 1-8 and 1-16 antigen dilutions were difficult to read.

The results were analyzed statistically and different antigen dilutions were found to contribute a highly significant degree of variation in this experiment. The log mean serum titers corresponding to the 1-2, 1-4, 1-8 and 1-16 antigen dilutions were 2.92, 3.27, 3.39 and 3.42, respectively; indicating a trend toward higher serum titers with higher antigen dilutions. Figure 2 presents a graph in which the log mean serum titers are plotted against the logarithms of the final antigen dilutions.

\[ \text{Log Final Antigen Dilution} \]

\[ \text{Log mean serum titer} \]

\[ 4 \text{fold dilution of antigen equals 2 fold average increase in serum titer} \]

The regular curve produced by connecting the points was not a consistent finding when this experiment was repeated. However, the straight line represents the linear regression of mean serum titer on antigen density or, in other words, the average change in serum titer produced by the dilution of the antigen. A fourfold dilution of antigen produced a twofold average increase in serum titer in this experiment. This finding was identical with
that obtained by Borg-Petersen and Fagraeus and was confirmed in repeated experiments. Since the antigen culture would have to be diluted with an equal amount of buffer to produce a twofold change in serum titers, it was considered questionable whether differences in antigen density would be a source of variation in the well regulated leptospirosis laboratory.

**Effect of Test Incubation Temperature**

The survey data (1) suggested that the incubation temperature of the agglutination-lysis test was a factor capable of producing significant variation in serum titer. Although the antigen-antibody combination would be accelerated by higher temperatures it was not so reasonable that the end point titer of the serum would be affected. Particularly would this be true if the time of incubation were of sufficient duration. The laboratories participating in the survey agreed almost unanimously on an incubation time of at least two hours. An experiment was designed to check this finding of the survey. A master set of fourfold dilutions was prepared using 16 serums. Three sets of tests were set up on the same batch of antigen. The first set was incubated at 23°C, representing an average room temperature. The second set was incubated at 30°C, and the third set at 37°C. All three sets were incubated for a period of exactly two hours and promptly read. An analysis of variance calculated on the data showed no significant effect of incubation temperature on the mean serum titers of the three sets of tests. However, the means for each temperature set increased from the lowest temperature to the highest. A plot of log serum mean titers and incubation temperatures is presented in Figure 3.

![Figure 3](image_url)

*Figure 3. Effect of Test Incubation Temperature

Sixteen sera on same antigen and dilution

Log Mean Titer

Temperature degrees Centigrade

F Temperature = 1.98 (Fₐₛ = 3.32)*
In order to show graphically that the increase in titer observed was of no importance, the grand mean was located by an arrow on the right side of the graph and the increment of one standard deviation was depicted above and below the grand mean. It was easily seen that the increase in serum titer over this temperature range was of no significance measured against the standard deviation of this experiment. It may be concluded the incubation temperature is not critical when the period of time permitted for the antigen-antibody reaction is at least two hours.

Effect of Serum Dilution Scheme

This experiment was planned to show the degree of variation introduced by the use of different dilution schemes. A large batch of antigen was tested against 17 serums. Three sets of dilutions were prepared with the serums: a tenfold scheme starting with a 1-10 dilution, a fourfold scheme starting with a 1-25 dilution, and a twofold scheme starting with a 1-25 dilution. All serums were carried to end point titers in all dilution schemes. The serum titers were transformed into logarithms and adjusted to a logarithmic midpoint between the 50 percent end point titer and the next dilution titer. From a logical point of view, stating a 50 percent end point titer was actually saying that the 50 percent end point occurred between the titer value specified and the next dilution titer employed. The data were analyzed and a summary is presented in Figure 4.

**Figure 4**

**Effect of Serum Dilution Schemes**

Seventeen sera on same antigen

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twofold</td>
<td>3.300</td>
</tr>
<tr>
<td>Fourfold</td>
<td>3.751</td>
</tr>
<tr>
<td>Tenfold</td>
<td>3.970</td>
</tr>
</tbody>
</table>

F Dilution Scheme = 34.07
(F.O = 4.88)
FACTORS IN AGGLUTINATION-LYSIS TEST

The three squares, one inside the other, indicate the extreme effect on serum titers produced by the use of different dilution schemes. The small black square represents the experimental error variance. The barred square depicts the magnitude the dilution scheme variance would have had to exceed to be highly significant, i.e., a value exceeded by chance only one percent of the time. The large white square shows the actual size of the dilution scheme variance and clearly indicates considerable effect on the serum titers in this experiment. The log serum means presented in the left side of the figure are all significantly different from one another and increase from twofold to tenfold. This effect is produced by the use of the same pipet in preparing serial dilutions. To reach the same end point titer the pipet is rinsed more times in the twofold scheme than in the tenfold scheme. The tendency of serum proteins to adhere to the wall of the pipet enhances the titer of a serum when the higher dilution scheme is employed. To further dramatize this phenomenon, Table 1 presents a comparison of the titers of five serums tested on the same antigen with a fourfold dilution scheme. The serums were diluted first, using a different pipet for each serial transfer, and second, using the same pipet for all of the transfers.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Different pipets</th>
<th>Same pipet</th>
<th>Increase in titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1,600</td>
<td>6,400</td>
<td>4 fold</td>
</tr>
<tr>
<td>9</td>
<td>400</td>
<td>6,400</td>
<td>16 fold</td>
</tr>
<tr>
<td>22</td>
<td>1,600</td>
<td>6,400</td>
<td>4 fold</td>
</tr>
<tr>
<td>23</td>
<td>1,600</td>
<td>25,600</td>
<td>16 fold</td>
</tr>
<tr>
<td>27</td>
<td>1,600</td>
<td>25,600</td>
<td>16 fold</td>
</tr>
</tbody>
</table>

It was observed that a four to sixteenfold difference in end point titer occurred when a different pipet was used for each mixing and transfer.

Experiment Combining the Effects of Antigen Strain, Dilution Scheme and Reader Variation on Twenty Serums in a Latin Square Design

The previous experiments gave clear indications as to the relative importance of the various factors studied. However, a valid criticism was offered that these experiments evaluated only one factor at a time, whereas in the field all types of permutations occurred. To overcome this objection, a large experiment was designed and executed in which all important factors varied simultaneously in a Latin square design. Four antigen strains of *L. pomona* commonly employed, Johnson, S-91, Clark and Pomona, were selected for the experiment. The antigen strains were tested against 20 serums daily for four successive days using two dilution schemes, fourfold and tenfold. An additional factor, reader variation, was introduced by using two test readers, EC and HS. The design of the experiment may be understood by studying Figure 5.
Experiment Combining Antigen Strains, Dilution Schemes, and Reader Variation on Twenty Sera in a Latin Square Design

<table>
<thead>
<tr>
<th>Antigen Strain</th>
<th>Fourfold</th>
<th>Tenfold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. C.</td>
<td>H. S.</td>
</tr>
<tr>
<td>Johnson</td>
<td>IV</td>
<td>I</td>
</tr>
<tr>
<td>S-91</td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>Clark</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Pomona</td>
<td>I</td>
<td>II</td>
</tr>
</tbody>
</table>

Antigen and dilutions prepared daily

Days are identified by the Roman numerals in the blocks. Master sets of fourfold and tenfold dilutions were prepared daily. As an example, on Day I, EC read a test prepared with the Pomona strain using the fourfold scheme and another test with the S-91 strain diluted tenfold. On the same day, HS read a set of tests on the 20 serums prepared with the Johnson strain in fourfold dilutions and the Clark strain in tenfold dilutions. At the end of the four days, tests made with each strain and dilution scheme had been read an equal number of times by the two readers.

The statistical analysis, which was necessarily involved, permitted the usual separation of components corresponding to antigen strain, dilution scheme, and reader. As a check, the experiment was performed three times on three different groups of serums. The interactions among the factors were studied, but no consistent effects were detected.

As would be expected from previous experiments, the tenfold dilution scheme consistently produced higher mean serum titers than the fourfold scheme (Figure 6). The difference between the two methods was highly significant.

Figure 6 presents a visual comparison of the error variance with the dilution scheme variance. The variance at the one percent probability level is also indicated. In all three experiments the dilution scheme variance greatly exceeded the variance at the one percent level.

It has been recognized that strains of the same leptospiral serotype vary in sensitivity against any given group of serums. These slight antigenic differences were further complicated in our experiment by the variations in the size of the cells. An additional problem was encountered in preparing
**Figure 6**

**Dilution Scheme Effect**

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment A</th>
<th>Experiment C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme</td>
<td>Mean</td>
<td>Scheme</td>
</tr>
<tr>
<td>4fold</td>
<td>3.85</td>
<td>4fold</td>
</tr>
<tr>
<td>10 fold</td>
<td>4.08</td>
<td>10 fold</td>
</tr>
</tbody>
</table>

**Figure 7**

**Antigen Strain Effect**

**Analysis of Variance**

- **Experiment I**
- **Experiment A**
- **Experiment C**

- Antigen strain variance
- Variance at the level of 1% probability
- Error variance
antigens of equal density from the four strains. The Johnson and Pomona strains grew more abundantly than the S-91 or Clark strains. It was necessary to dilute the former strains with buffered saline in order to reduce them to comparable levels of density. Even so, the cells of the S-91 and Clark strains were uniformly smaller in size. Standardization of the antigens was achieved by dark-field microscopy. As a check, a series of five counts using the Petroff-Hausser Bacteria Counter [C. A. Hausser and Son, Philadelphia, Pa.] were made during the experiments. Although the counts were low when compared with the recommended standard, there were no significant differences among them which could be accused of affecting antigen sensitivity.

The use of the four antigen strains produced as large a component of variation in the serum titers as the use of different dilution schemes. In Figure 7 are presented graphically the relative sizes of the antigen strain variances as compared with the experimental error variance for each experiment.

In all three experiments the antigen strains introduced a highly significant degree of variation. Of even greater interest was the fact that the sensitivity of the four strains was consistent throughout the three experiments. Figure 8 presents a graph of the log mean serum titers for each antigen strain by experiment.

**Figure 8**

**Antigen Strain Effect**

*Comparison of means of 80 Sera*

Log Antigen Means

Experiments

antigen means not significantly different by Q test
The lines connecting the points do not cross, indicating clearly that the comparative degree of sensitivity of each antigen strain remained the same. The highest mean titers were shown by the Clark strain, followed by the S-91, Pomona, and Johnson strains in order of decreasing sensitivity. Further study of the data was made to determine whether the antigen means were significantly different at the five percent level using the Q test (9). All of the differences among antigen means were significant except those between the Johnson and Pomona strains in experiments A and C. Granted that adequate standardization of the density of the antigens was obtained, it must be concluded that the use of different strains of *L. pomona* introduces an important component of variation into the agglutination-lysis test.

Although the reading and interpretation of agglutination-lysis tests would be expected to vary from one test reader to another, the two workers who participated in these experiments had made an effort to standardize their readings approximately one year previously. The results were somewhat unexpected. The reader variance in the first two experiments was insignificant. However, in the third experiment a considerable divergence of test interpretation appeared. Figure 9 reveals this finding in the usual manner.

**Figure 9**

*Reader Effect*

*Analysis of Variance*

*Experiment I*  *Experiment A*  *Experiment C*

- Reader variance
- Variance at the level of 5% probability
- Error variance

The unexpected finding in experiment C might be charitably blamed on an unusual chance occurrence. However, a more accurate appraisal of the data would be to say that the individual reader's interpretation of end points varies from day to day. In measuring reader effect on the test, a component of variation was studied which was not a fixed attribute such as that of antigen strain. A mental picture of a 50 percent end point must vary within one individual reader over a period of time so that this factor is a source of variation which can never be completely controlled.
Several experiments were performed on the agglutination-lysis test to determine which factors in the protocol of the test could be altered so as to produce significant changes in serum titers. Some of the factors studied which were not considered to be important sources of variation were test incubation temperature, standardization of antigen, and serum dilution preparation. Other factors in the protocol were found to be quite sensitive. The use of different dilution schemes, antigen strains, and test readers effectively altered serum titers. Antigen density was found to be a serious source of variation only when the changes in density were extreme. The results of the experiments described stress the need for the adoption of a standard method of performing the agglutination-lysis test by all laboratories engaged in either the research or diagnosis of leptospirosis.

ACKNOWLEDGMENTS

The author wishes to thank Dr. H. O. Hartley of the Statistics Department of Iowa State University for his advice and assistance in analyzing the data and Dr. J. E. Williams of the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Washington, D. C., for supporting the project. The participation of Mrs. Helen Sailsbury in the experiment involving reader variation and her excellent technical assistance throughout the work are gratefully acknowledged.

REFERENCES

AN EPIZOOTIC OF LEPTOSPIROSIS IN INSTITUTIONAL HERDS OF CATTLE AND SWINE

DAVID E. HUGHES, D.V.M., M.S. AND HERBERT L. KEECH, B.S.

During the late summer and autumn of 1958 an epizootic of leptospirosis, that was due to *Leptospira pomona*, occurred in five herds of cattle and one herd of swine at the Agricultural Research Center at Beltsville, Maryland. A study of this epizootic is significant for the following reasons:

1. The herds had been under surveillance for two years so that a background of serological information was available before the disease appeared.
2. No animals were vaccinated and no cases of leptospirosis had been recognized during the previous herd history.
3. Neither vaccination nor therapeutic treatment was used to interfere with the nature and course of the disease.
4. Any animal was available for more detailed study.
5. The study offered an opportunity to develop definitive data that would be difficult to acquire under ordinary field conditions.

MATERIALS AND METHODS

The five herds of cattle and the one herd of swine are individually described under results to maintain continuity of the presentation.

The agglutination-lysis (A-L) test was used exclusively. Serum dilutions were prepared in tenfold series resulting in a dilution of 1:20 in the first tube after addition of the antigen. *Leptospira pomona* (Johnson) culture grown in a seven percent rabbit serum enriched Stuart’s (1) medium for 72 hours at 29°C. was used as antigen. Readings were graded as negative for no clearing or graded as 1, 2, 3, or 4 to correspond with 25 percent, 50 percent, 75 percent, or 100 percent relative clearing, respectively. A reading of one was considered to be positive for any given dilution.

Urine, blood, or tissues were cultured in Chang’s (1) semisolid medium and inoculated into guinea pigs. Heart blood for culturing was collected from the guinea pigs on post inoculation days five and seven and any time the animals became febrile. The A-L test was made on the guinea pig blood during the third week after inoculation. Rectal temperatures of the guinea pigs were recorded daily for 12 to 14 days. Cultures were incubated at 29°C and examined weekly with a dark field microscope for leptospires.

Infected cattle were segregated on the premises of the various units by holding them in box stalls apart from the remainder of each herd. Disinfectant
dips were used for boots and tools and access to the stalls was limited. Some animals were transferred to isolation facilities at the Animal Disease Station (ADS) for detailed cultural and post-mortem examination.

Infected swine were separated from the rest of the herd and disposed of whenever possible. Some of these animals were transferred to A.D.S. for further studies.

No cattle were moved between these herds and to our knowledge there was only a limited contact between personnel or exchange of vehicles.

Beginning in 1956 blood samples were collected annually from all cattle, which were more than 30 months of age. New animals entering the various herds were quarantined and tested before being admitted. Special collections were made from individual cows after calving or after any abnormal termination of pregnancy. Furthermore, when leptospirosis was either suspected or detected in any particular group of each herd, all animals in that group, regardless of age, were bled at intervals ranging from four to 21 days.

**RESULTS**

Herd No. 1 was assembled between October of 1957 and February of 1958 in three groups. Each group consisted of 13 Ayrshire, 13 Brown Swiss, and 14 Holstein heifers; all were approximately nine months of age. The animals originated from individual farms in Kansas, Ohio, and New England. The animals were not tested for leptospirosis before arriving at Beltsville. The Ohio group, which arrived during October, was tested serologically and found to be free from reactions to *L. pomona*. The Kansas group arrived during November and was also tested against *L. pomona*. Titers ranging from 200 to 200,000 were noted in seven animals, which were immediately separated from the rest of the group (Table 1). The seven animals, as well

<table>
<thead>
<tr>
<th>Heifer Number</th>
<th>11/5/57</th>
<th>11/25/57</th>
<th>1/3/58</th>
<th>2/3/58</th>
</tr>
</thead>
<tbody>
<tr>
<td>214</td>
<td>200</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>2,000</td>
<td>200</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2,000</td>
<td>200</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>415</td>
<td>20,000</td>
<td>20,000</td>
<td>2,000</td>
<td>2,000</td>
</tr>
<tr>
<td>24</td>
<td>200,000</td>
<td>200,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>424</td>
<td>200,000</td>
<td>200,000</td>
<td>20,000</td>
<td>20,000</td>
</tr>
<tr>
<td>420</td>
<td>200,000</td>
<td>200,000</td>
<td>200,000</td>
<td>200,000</td>
</tr>
</tbody>
</table>

as the rest of the Kansas group, were tested 20, 39, and 59 days later. By March, the titers of four of the seven had receded to 20 or lower; they were then returned to the herd. The three that continued to react were transferred to A.D.S. Attempts at isolation of leptospires from urine and body tissues of the three were unsuccessful. The New England group arrived in February
of 1958 and, when tested, three animals showed a slight reaction at the 1:20 dilution. The entire herd of 120 was tested again in October. One animal with a titer of 200,000 was detected but subsequent attempts to culture leptospires were unsuccessful. Several subsequent tests revealed no animals with titers of 200 or higher. There was, however, a relatively large number of animals with titers of 20 throughout the 18-month testing period. These titers were in most cases transient and were seen in 78 of the 120 animals.

Herd No. 2 consisted of twin Angus calves acquired at weaning time from individual farms. A group of 16 calves that had been collected from Virginia, West Virginia, and Maryland was tested on arrival in August of 1958. One set of twin calves reacted at 1:20,000 dilution with *L. pomona* antigen. A second test made 23 days later detected another set of twins that also reacted at 1:20,000 dilution. All four calves were segregated from the remaining animals. Urine from the four animals was inoculated into guinea pigs at weekly intervals for seven weeks. *Leptospira pomona* was isolated from guinea pigs inoculated with urine from two of the four calves. Isolations were made from one animal on day 37 and the other animal on days 29, 37, and 45. Nothing of significance was observed in the remaining calves.

Herd No. 3 consisted of nonlactating pregnant cows that were pastured in a field adjacent to Herd No. 1. As these cows calved they were returned to the milking herd, which consisted of approximately 340 cattle. On September 19, 1958, one cow aborted in the pasture. The fetus was partially consumed and was not suitable for bacteriological examination. The cow was removed from the group and a blood sample submitted for serological examination. The initial test revealed a titer of 200,000 against *L. pomona* antigen. Urine was collected and inoculated into guinea pigs with negative results. The remaining 50 pregnant cows in the pasture and the 17 cows that had been returned to the milking herd during the previous four weeks were tested. One pregnant cow showed a titer of 20,000 and was segregated. Attempts to isolate leptospires from her urine were unsuccessful, and a serum titer of 2,000 persisted for 18 months. The herd was tested seven, 28, 50, 77 and 97 days after the first test. The second test revealed no new titers. The third test detected a titer of 20,000 in a cow that was previously negative. This animal was segregated and aborted 10 days later. *Leptospira pomona* was isolated from guinea pigs inoculated with urine collected on the day of abortion and again on day 10 after abortion. The fourth test revealed 1 cow that had a titer of 2,000; leptospiral isolation attempts from her urine were unsuccessful and her A-L titer rapidly declined after reaching 20,000 which receded to 20 within 42 days. On the fifth test another cow had an initial titer of 2,000 that receded to 200 within 23 days. The sixth test revealed nothing of significance.

Herd No. 4 contained 158 dual purpose cattle. In early November of 1958, the herd had been moved from the pasture into the barn area for winter housing. At that time, the animals were grouped according to milking status, pregnancy status, and age. A herd test on November 18 revealed one cow
that had a titer of 200,000. Additional tests were made on the animals, including those under 30 months of age, and a total of 20 females with significant A-L titers were found in the following sequence: one in November, 10 in December, six in January, and three in February. Eight were pregnant and four aborted during December. Guinea pigs were inoculated with urine from five animals and *L. pomona* was isolated from two, both of which had aborted. In retrospect, the first nine that developed titers, including the four that aborted, had been in the same pasture until early November. The remaining 11 were scattered in the various groups that had been formed after removal from the pastures.

Herd No. 5 had been maintained as a herd free of specific pathogens until the results of a routine test on January 14, 1960, revealed two animals with a titer of 200,000 against *L. pomona*. Thirteen days later a third animal was found with a titer of 200 which did not go higher. *Leptospira pomona* was isolated from urine collected on January 29 from one of the two animals that had a titer of 200,000. No subsequent isolations were made and no additional reactors were detected.

In the swine herd, the young animals were bled as soon as they were taken off experimental status. Gilts and sows that failed to farrow or were observed to abort were tested as the occasion arose. The remaining sows were bled at weaning time. The first evidence of infection came from the A-L test of serum from two sows that aborted in February 1959, both of which had titers of 200,000 for *L. pomona* antigen. A total of nine sows aborted; five of these had titers of 2,000 or higher against *L. pomona* when tested a few days after abortion. One was negative at 1:20, one positive at 1:200 and two were not tested. Altogether 773 serums were tested of which 63 (34.1 percent) of 184 sows and six (40 percent) of 15 boars were positive at 1:200 or higher. *Leptospira pomona* was isolated from the kidney tissues of two sows by direct culture, but no isolations were made from guinea pigs inoculated with the tissues from the same source. The only clinical sign observed was abortion. As soon as the diagnosis was made, the entire herd of swine was vaccinated with commercial *L. pomona* bacterin. During the past 18 months, no evidence of new infection has been found in this unit; however, the role of vaccination in controlling the spread of infection could not be evaluated from the data available.

In June of 1959, a test was made on 548 serums from cattle in herds one and three against 11 leptospiral serotypes. One hundred thirty-seven (24 percent) were negative at 1:20 with all of the antigens. A summary of the positive reactions and a listing of the serotypes used are presented (Table 2). A complete breakdown of reactions noted in the 26 cows that reacted at a dilution of 1:200 or higher against one or more antigens is also presented (Table 3). Although the herds had been exposed to *L. pomona* infection six to eight months previously, antibodies against this serotype were not predominant. More than four times as many reactions to other serotypes were found. Positive reactions to *Leptospira ballum* exceeded those to *L. pomona* in both the 1:20 and 1:200 dilution. *Leptospira autumnalis* and *Leptospira*
AN EPIZOOTIC OF LEPTOSPIROSIS IN CATTLE AND PIGS

TABLE 2

Agglutination-lysis reactions in 548 bovine serums tested against 11 leptospiral serotypes

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Antigen</th>
<th>Strain</th>
<th>1:20 Dilution</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>L. pomona</td>
<td></td>
<td>Johnson</td>
<td>163</td>
<td>29.8</td>
</tr>
<tr>
<td>L. australis A.</td>
<td></td>
<td>Ballico</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>L. autumnalis</td>
<td></td>
<td>Akiyama A</td>
<td>113</td>
<td>20.3</td>
</tr>
<tr>
<td>L. ballum</td>
<td></td>
<td>S-102</td>
<td>232</td>
<td>42.0</td>
</tr>
<tr>
<td>L. canicola</td>
<td></td>
<td>Ruebush</td>
<td>68</td>
<td>12.4</td>
</tr>
<tr>
<td>L. grippotyphosa</td>
<td></td>
<td>Andaman</td>
<td>120</td>
<td>21.9</td>
</tr>
<tr>
<td>L. hebdomadis</td>
<td></td>
<td>LT-117</td>
<td>24</td>
<td>4.3</td>
</tr>
<tr>
<td>L. hyos</td>
<td></td>
<td>—</td>
<td>2</td>
<td>0.36</td>
</tr>
<tr>
<td>L. icterohemorrhagiae</td>
<td></td>
<td>Wijnberg</td>
<td>106</td>
<td>19.3</td>
</tr>
<tr>
<td>L. sejroe</td>
<td></td>
<td>K. M.</td>
<td>28</td>
<td>5.1</td>
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<tr>
<td>L. bataviae</td>
<td></td>
<td>Van Tienen</td>
<td>15</td>
<td>2.7</td>
</tr>
</tbody>
</table>

1 One serum positive at 1:2000.

icterohemorrhagiae reactions exceeded or equalled those to L. pomona in the 1:200 dilution, but not in the 1:20 dilution.

In January of 1960 one heifer (4692), which was located in the same field where infection was previously found in herd No. 5, had a serum titer of 200 with L. pomona. Fifteen days later, the titer of this animal had receded to 20. When the serum of this heifer was tested against the remaining 10 serotypes (Table 4), it was evident that L. icterohemorrhagiae was the offending organism. This prompted a search for the source of the infection. Wild animals were trapped in the immediate vicinity of the pen area. Three strains of leptospira were isolated from rats and were tentatively identified as L. icterohemorrhagiae. One strain from the kidney of an opposum was believed to be L. ballum. A fifth strain from the kidney of a raccoon appeared to be L. grippotyphosa. Leptospira ballum was isolated from two of six house mice trapped in the No. 3 herd pasture during October of 1958. These isolations prove that wild animals in the vicinity of these cattle were carrying these serotypes. As previously mentioned, antibodies against these same serotypes were found in the serums of 26 cattle from herds No. 1 and No. 3.

DISCUSSION

In this study, the reliability of the serological basis for diagnosis of leptospirosis was reaffirmed by the isolation of L. pomona from animals with titers. However, individual differences in level and duration of titer emphasize the limitation of the method (Table 1). Low level titers, 20 and 200, could have resulted from (a) subinfectious exposure, (b) residual effect of earlier infection, and (c) infection with heterologous serotypes. Other titers may have been caused by active infection, but the higher titer level did not necessarily indicate either the existence of leptospiruria or the time
TABLE 3
Reactions of 26 Bovine Serums Against 11 Leptospiral Serotypes

<table>
<thead>
<tr>
<th>Animal Serum No.</th>
<th>Pomona</th>
<th>Australis</th>
<th>Autumnalis</th>
<th>Ballum</th>
<th>Canicola</th>
<th>Grippotyphosa</th>
<th>Icterohaemorrhagiae</th>
<th>Sejroe</th>
<th>Batavia</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>200</td>
<td>-</td>
<td>200</td>
<td>20</td>
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<td>-</td>
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<td>-</td>
<td>200</td>
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<td>-</td>
</tr>
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<td>20</td>
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</tr>
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<td>200</td>
<td>-</td>
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* All serums negative at 1:20 with *L. hebdomadis* and *L. hyos.*

† Number is reciprocal of highest reacting dilution.

‡ — — Negative at 1:20:

of infection. The rapid loss of titer noted in certain cows may have been due either to the failure of the infection to develop through the leptospiremia and subsequent localization stages (4) or to infection with a heterologous serotype. The persistence of titer could not be adequately explained although it could have been due to a localized focus of infection in the body—a fact which has yet to be proved.

Three possible sources of infection in these herds were postulated. First, it was introduced through purchase of infected animals in two herds. Second, the infection in these two herds created the possibility of introduction into the other herds. Theoretically, this was possible but the limited secondary infections, the practice of segregation, and the limited contacts between herds argue against this postulation. Third, a wild animal source of infection was
AN EPIZOOTIC OF LEPTOSPIROSIS IN CATTLE AND PIGS

TABLE 4

Agglutination-lysis Titer of Serum From Cow 4692 Tested Against Five* Leptospiral Serotypes

<table>
<thead>
<tr>
<th>Leptospiral Serotype</th>
<th>Day</th>
<th>Ballum</th>
<th>Canicola</th>
<th>Icterohaemorrhagiae</th>
<th>Pomona</th>
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* = Negative at 1:20.

* Negative to six additional serotypes, see Table 2.

suggested by studies in Georgia (2) and Louisiana (3). Wildlife was abundant in this area of Maryland and ample opportunity existed for contact with both cattle and swine. The presence of cattle serum titers against serotypes isolated from wild animals supports this suggestion (Table 3). It must be noted, however, that a wild animal reservoir of *L. pomona* was not identified.

Probably the spread of infection was limited by frequent testing and segregation of animals with high or rising titers. The possible exception noted in herd No. 4 could be explained by the failure to test all animals initially and the failure to retest frequently enough. Five cows aborted after the disease was found in their respective herds; however, four were infected when the herd diagnosis was made. If this herd had been vaccinated, only one cow might have been given protection, and no benefit would have been expected in those infected or exposed before vaccination. Similar circumstances exist under field conditions where extensive vaccination is done.

No special features distinguished the leptospiral abortions from those which occurred from other causes. Only six of 13 abortions in herds No. 3 and No. 4 could be attributed to leptospirosis. In swine as in cattle, not all the abortions occurred in sows that reacted serologically. A positive serological reaction in a previously negative animal could be significant, but in the absence of a serological history it must be remembered that residual high titers may be present for several months and abortion from other causes could be erroneously diagnosed as leptospirosis. Whenever a series of abortions is encountered, each should be considered a separate diagnostic problem until proved otherwise.

Testing with 11 serotypes revealed a different picture of leptospirosis than when only *L. pomona* was used. Although no disease was seen in these cattle, the significance of their titers should not be overlooked. Isolation of three serotypes from wild animals and *L. pomona* from the cattle herd was one
indication of the specificity and significance of the titers. Furthermore, it indicated that any study of bovine leptospirosis should include ecological investigation. Specifically we need to know whether these other serotypes can produce disease in cattle, and what effect exposure to these serotypes may have upon the course of infection with *L. pomona*.

**SUMMARY**

A study of leptospirosis was made in five herds of cattle and one herd of swine. A total of 40 cattle developed agglutination-lysis (A-L) titers; 14 of these were pregnant and six aborted. *Leptospira pomona* was isolated from the urine of six cows including three that had aborted. A total of 69 swine developed A-L titers; 63 were bred sows and five of these aborted. *Leptospira pomona* was isolated from kidney tissues of two sows that had aborted.

Infection was introduced in two herds by newly purchased cattle and in three cattle herds and in the swine herd by an unknown manner.

Spread of infection was believed to have been controlled by repeated testing and segregation of cattle with high or rising titers.

Antibodies were found in cattle serum when tested against 11 leptospiral serotypes. The possible significance of five serotypes was suggested by titers of 200 and also by isolation of four of these five from wild and domestic animals.

**ACKNOWLEDGMENT**

The authors wish to express their appreciation to Dr. G. E. Whitmore, Veterinarian, Animal Husbandry Research Division, and to Dr. H. C. Ellinghausen, Microbiologist, Animal Disease and Parasite Research Division, for their assistance in the conduct of this study.

Appreciation is also expressed to members of the Animal Husbandry Research Division whose cooperation made this study possible.

**REFERENCES**

REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

E. Roth, Chairman, Baton Rouge, Louisiana; E. H. Bohl, Columbus, Ohio; H. S. Bryan, Kalamazoo, Michigan; R. J. Byrne, Silver Spring, Maryland; E. A. Garbrey, Ames, Iowa; D. E. Hughes, Beltsville, Maryland; S. G. Kenzy, Pullman, Washington; E. V. Morse, Lafayette, Indiana; W. L. Sipple, Kissimmee, Florida; L. W. Turner, Nashville, Tennessee; C. York, Indianapolis, Indiana.

Previous reports of this Committee dealt with the diagnosis, prophylaxis, and control of leptospirosis. The basic concepts of these reports remain unchanged although a few minor adjustments should be recommended to bring them up to date.

Serologic procedures are most commonly used as an aid in the diagnosis of leptospirosis. Detailed procedures for the performance of the Stoenner and Galton plate tests were outlined last year. The agglutination-lysis test was similarly detailed. It is repeated here with minor revisions to simplify the procedure.

1. The agglutination-lysis test
   a. The following system of serum dilution should be used.
      (1) Prepare serum dilutions of 1:50, 1:500, 1:5,000 and 1:50,000.
      The following scheme of dilution is suggested as a guide:
      \[
      \begin{align*}
      \text{0.4 ml serum} & + \text{1.6 ml diluent} = 1:5 \\
      \text{0.2 ml serum} & + \text{1.8 ml diluent} = 1:50 \\
      \text{0.2 ml serum} & + \text{1.8 ml diluent} = 1:500 \\
      \text{0.2 ml serum} & + \text{1.8 ml diluent} = 1:5,000 \\
      \text{0.2 ml serum} & + \text{1.8 ml diluent} = 1:50,000.
      \end{align*}
      \]
   After mixing an equal amount of each serum dilution with antigen, the final dilutions are 1:100, 1:1,000, 1:10,000 and 1:100,000.
   (2) Dilutions should be prepared using Screnson’s phosphate buffer at PH 7.4 containing 0.85 percent sodium chloride; and
   (3) Use a one ml. serological pipette.
   b. The test should be inculcated for two hours at room temperature.
   c. The reaction should be estimated as the degree of clearing when compared to the antigen control (one volume antigen plus one volume of diluent). A 50 percent reaction should be considered as positive for the particular dilution. To gain an impression of the appearance of a 50 percent reaction, a reference can be prepared by mixing one volume of the antigen with three volumes of diluent.
   d. Antigen used for the test should be:
      (1) The type strain \textit{L. pomona} (Pomona).
      (2) Grown in Stuart's medium enriched with 7-10 percent rabbit serum.

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Three to five day old cultures started by inoculating with 10 percent of an actively growing culture incubated at 28-30°C. Cell counts as determined using a Petroff-Hauser counting chamber should fall within a range of 175 to 225 million organisms per milliliter.

When the culture requires dilution, use uninoculated Stuart's medium.

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

f. Both negative and positive serum controls should be used at the same dilutions as the test.

Two serological methods of more recent origin, fluorescent antibody technique and the hemolytic test, are still in the process of refinement. The Committee continues to observe these developments with interest.

Culture procedures are seldom used in the diagnosis of leptospirosis. Direct isolation of leptospires in culture medium is equivalent to using laboratory animals from the standpoint of simplicity, time and sensitivity. This method is applicable to diagnostic laboratories where other bacteriological diagnoses are made.

Fletcher's semisolid medium (Difco) is recommended. Three to five drops of blood aseptically collected during the febrile stage should be inoculated into each of four tubes of medium. Urine collected three-four weeks after acute signs or at the time of abortion should be diluted with nine parts of Stuart's medium (Difco) immediately after collection. Following transport to the lab a series of tenfold dilutions extending through five tubes should be prepared. Two to three drops of each dilution inoculated into two tubes of Fletcher's medium. Tissues such as kidney collected aseptically soon after death should be ground into a 10 percent suspension (in Stuart's medium). The dilution and inoculation should be made as described for urine. Liver, kidney and pericardial fluid are considered to be the tissues of choice when culturing aborted swine fetuses. Cultures should be incubated at 28°C -30°C for six weeks. Dark field examination at 3-600x should be made at weekly intervals. Isolations should be submitted to a reference laboratory for typing.

The position regarding the use of L. pomona bacterins in cattle and swine has not changed from previous reports of this Committee.

L. pomona is still recognized as the predominant cause of leptospirosis in cattle and swine. L. canicola has been isolated from cattle and swine. L. hardjo has been isolated from cattle and may be responsible for the widespread occurrence of antibodies in cattle serum being detected with L. sejroe antigen. This prompts the suggestion that L. hardjo be used instead of L. sejroe as the test antigen. Serological evidence suggests the possibility of infection of cattle with other serotypes. Proof of this must await isolation and identification of additional serotypes from livestock. At the present time vaccination of cattle and swine with serotypes other than L. pomona is not justified.
Leptospirosis in wildlife is being studied more intensively in several areas of the United States. Isolations of *L. pomona* have been made from skunks, raccoons, bobcats, opossums, dogs, deer, a woodchuck and a red fox. Preliminary investigations in Georgia and Louisiana indicate that the incidence of *L. pomona* infection in skunks is significantly higher than in other animals mentioned. They may be of importance as a source of infection for livestock.

It is premature to consider a legislative regulatory control program for leptospirosis on a national, area, or state basis. The presence of *L. pomona* infection in wild animals emphasizes that the disease is not limited to domestic animals, hence it cannot be controlled by quarantine.

Increased support for research in the diagnostic, immunologic, chemotherapeutic and epizootologic aspects of the disease is urgently required for a better understanding of leptospirosis.
POULTRY DISEASE CONTROL IN INTEGRATED OPERATIONS

J. L. ROUNTREE, D.V.M., M.S.

Augusta, Maine

The remarkable growth of the integrated poultry industry has opened fertile ground for the veterinary epizootiologist. This growth, generally without careful thought to disease prevention practices, has placed poultry diseases on a panzootic basis. No area of the United States can lay solid claim to freedom from the major poultry diseases. And yet, the industry has grown and generally prospered.

The poultry industry is still growing, but it appears that the problems associated with disease are growing faster. There is a narrow economic margin in the industry and with this narrow margin, clinical and subclinical disease conditions assume a position of greater importance in relation to production costs. Farm production efficiency and plant condemnations are serving to illustrate the need for a critical look by the integrator at disease control methods.

Unfortunately the veterinarian has not carried his share of the burden in the field of poultry diseases. Except for a relative handful of avian pathologists, the field of poultry diseases has been ignored and disparaged. This has allowed the industry to expand without an equal expansion of applicable disease control knowledge. The poultry management specialist and the nutritionist who have found it necessary to assume the bulk of the practical disease control effort have made a solid contribution, but they recognize as the veterinary profession should, that an intensive cooperative effort is a necessity of growing importance to the poultry industry.

The control of poultry diseases must by the nature of integration vary from area to area in this country. The control measures which apply to New England will not apply directly to conditions prevalent on the Delmarva peninsula. And yet, the basic principles of disease control are the same in any area. It is therefore the problem of the responsible personnel to develop methods based on sound principles which will function for a particular integrator or area.

Prevention of disease occurrence by restriction of transmission cycles must be the basis of developing a sound disease control program in an integrated operation. Each disease condition offers a different picture, but will fall within the working frame of one, or a combination of the following preventive or transmission cycle restricting methods: A. Environmental or physical control. B. Vaccination and C. the use of preventive medicaments.

No one of the above methods will stand alone, but the environmental method must be considered as the keystone in building a sound prevention program. The depth or effectiveness, which can be developed through
environmental or physical control will determine to what extent the other methods of prevention must be employed. The integrator who fails to develop a sound physical disease control program on which can be built necessary vaccination and preventive medicament programs will find tough going in the increasingly competitive poultry industry.

The possible effectiveness of physical or environmental disease control will vary with industry concentration in an area and conditions inherent to the particular integrator. However, nowhere has the potential of an environmental or physical disease control program been approached. House and equipment sanitizing, cleanliness of personnel between farm visits, controlled building environment and education of poultry raisers are but a few factors in need of intensive exploitation—to the over-all benefit of the poultry industry.

The extent to which vaccinations and medicaments are used in a preventive program depends on the effectiveness of the environmental control. It is axiomatic that a disease break, which never occurs because of preventive methods, costs the poultryman and the industry less by far than the conventional wait for trouble and treat approach. Even though the preventive approach to disease control is unquestionably correct, care must be exercised when designing preventive programs so that they are economically as well as theoretically sound.

Diagnosis of disease conditions is twofold in purpose in an integrated operation. First, diagnosis provides the basis for sound therapeutic recommendations and second it provides information necessary for the effective development of the preventive approach. In using therapeutics, over-all cost and return on the investment is the prime consideration. These considerations encompass both the effect on the production efficiency of the flock and the production efficiency of the processing plant. In an integrated operation it becomes difficult to draw a clearly defined line between investment and return when both the flock, and plant factors must be considered. Difficult or not, therapeutic recommendations must be rendered on consideration of economics alone and at times under somewhat emotional circumstances.

Disease control in an integrated operation is largely a problem or challenge within the particular firm. However, the over-all disease potential of a state or area must be recognized in light of the effect it will have on a single integrator. The conditions vary from area to area, but in any case sound working relationships must be established between the integrator and state agencies having responsibility and interest in disease control. Over-all area or state disease control programs, which reduce the disease potential on a sound basis also reduce the probability of disease challenge to an individual or to an integrator.

In summary, it is obvious that a great number of factors must enter into planning and implementing a sound disease control program for an integrator. Primary on the list of factors is the extent of cooperative effort developed by management specialists, nutritionists and veterinarians. Through cooperative effort, a sound disease prevention program promulgated on environmental control and supplemented by vaccination and medicaments can be established.
Diagnosis of diseases will aid in illuminating areas in need of preventive attention and provide a sound basis for therapeutics. Preventive methods and therapeutics have a single reason for existence economics. The ease of developing satisfactory inter-company control programs can be aided by integrator cooperation in over-all area or state disease control work. Finally, it should be pointed out that the vacuum of knowledge relative to on the farm poultry disease control can be reduced only by more workers in the field in cooperative effort with all interested disciplines.
REPORT OF THE COMMITTEE ON DISEASE CONTROL
IN LIVESTOCK INTEGRATION

A. L. Sundberg, Chairman, Des Moines, Iowa; E. L. Brower, Trenton, New Jersey; F. G. Buzzell, Augusta, Maine; J. G. Flint, St. Paul, Minnesota; F. S. Honsinger, Juneau, Alaska; J. W. Mann, Atlanta, Georgia; J. E. Stuart, Sacramento, California; W. M. Thompson, Phoenix, Arizona.

In keeping with the method previously employed, to gather information pertinent to disease control in livestock integration, your Committee again submitted questionnaires to each of the Livestock Sanitary Officials of their respective state or territory.

Of the 52 questionnaires submitted, 38 were completed and returned; this was a 74 percent response as compared to 83 percent response the year preceding.

It is interesting to note how closely the replies coincide with those of a year ago. Twenty-one states as compared to 26 in 1959, reported integrated operations in one form or another, involving one or more classes of livestock, with integration ranging from partial to virtually complete.

Complete integration appears to be confined mostly to the poultry industry or more specifically the broiler industry.

Seventeen states reported integration as non-existent, compared to 14 states reporting similarly in 1959.

Seven states reported an increase, or rather an expansion of existing integrated operations, whereas seven states reported integration as having stabilized or actually diminished.

Five states reported an increase in disease control problems due primarily to inefficient management and general lack of sanitation, or resulting from laymen employing “do it yourself” veterinary tactics in conducting testing and/or vaccination programs, normally under state supervision.

Nineteen states reported no increase in disease control problems, with a few of the 19 states reporting disease control problems as actually reduced, due to a lesser number of managers (or producers) to contact; better management and sanitation practices; and a greater tendency on the part of the integrator to protect his financial investments.

None of the 38 states reporting has specific regulations covering integrated operations; however, a greater majority of the states indicate they have ample existing statutory provisions to control such operations from a disease standpoint.

Based on the data accumulated over two consecutive years it would appear that integrated operations in livestock production have presented no serious disease control problems, other than those which would normally be handled on a state level.
It would appear that livestock integration presents more of an economic problem to the livestock producer than it does a disease control problem.

Therefore; Since integrated operations in livestock have to date presented no serious disease control problems, and;

WHEREAS: Any disease control problems resulting from such operations would normally be handled on a state level, and;

WHEREAS: All resultant transmissible disease problems would fall within the province of the various existing committees on transmissible diseases of livestock;

Your Committee recommends that the Committee on Disease Control in Livestock Integration be abolished, and the functions of said Committee be absorbed by the appropriate Committee on Transmissible Diseases.
I. D. PORTERFIELD, Ph.D. and W. E. PETERSEN, Ph.D.

University of Minnesota, St. Paul, Minnesota

As late as 1892, Ehrlich (1) showed that immunity was acquired and not inherited. He also showed that antibodies were transferred to nursing mice through milk. For example, he took litters of two mice, one immunized to Ricin (a plant protein) and the other not, were exchanged. After the normal litter had nursed the immune mother for 23 days, they were then injected with 11X, the lethal dose of the specific antigen and gave only a slight indication of a local reaction. After repeating similar experiments, he concluded that milk, as such, is able to transmit antibodies to a nursing organism and give it immunity that increases with the duration of nursing.

You are familiar with Smith and Little's experiment (2) where they took two groups of calves, one group of 10 receiving colostrum after birth and the other group of 12 calves receiving normal whole milk. All of the colostrum-fed calves survived and eight of the 12 not receiving colostrum died.

Ingraham et al. (3) report that of 161 calves fed colostrum, 118 survived, while in a group of 103 calves that did not receive colostrum only nine survived. They concluded that colostrum contains protective substances against potential pathogens prevalent in the neighborhood in which the dam has been living.

Giltner, Collidge and Huddleson (4) found that when a pure culture of Brucella abortus was introduced into the cistern of the cow's udder, agglutinins for this organism appeared in the same quarter the following day, after which they appeared successively in each of the other quarters and finally disappeared. They believed that antibodies were produced locally in the cow's udder.

Mitchell, Walker and Bannister (5) infused into active bovine mammary glands live Newcastle, Duck, and Influenza Virus. They found that the virus propagated in the infused quarter two weeks reached a titer of $10^6$. During this time, no titer was found in milk from the other three quarters nor in the blood serum. Soon after the first two weeks antibodies were found in milk from the infused quarter, a few days later in the blood, and eventually in the milk from the other quarters.

More recently, we designed experiments to obtain answers to some of the many questions raised about antibody response to antigens introduced systemically or directly into the cow's mammary gland. Specifically, our study attempted to (1) shed more light on the formation of antibodies in the bovine mammary gland, (2) on the speed of response in the udder to locally introduced antigens, and (3) on the effect of varying methods of
introducing the antigen, such as intramammary and systemic, upon antibody content of the milk. In addition, data were collected on the booster effect of repeated administration of the antigen by the various routes and its effect upon the health of the animal and the mammary gland, and finally the rate of disappearance of the antibody from both milk and blood.

METHODS AND MATERIALS

Registered Jerseys and grade cows were treated with phenolized Salmonella pullorum antigen standardized to 40 to 80 billion cells per ml. Only cows initially showing negative titers in their blood serum and milk (if lactating) were used. The antigen was administered in one of three ways: (1) By subcutaneous injections made in the neck, or (2) by intramuscular injections made in the rump near the thurls, and (3) by intramammary infusions made through the teat meatus with a syringe and teat cannula. Prior to the infusion, a solution of 400,000 units of penicillin and one gram of streptomycin was introduced into the udder as a prophylactic measure.

An assay for antibodies was made on the whole milk and the clear blood serum of all experimental animals, before treatment and at frequent intervals after the antigen had been administered. The assay was done by plate agglutination using the same vaccine and examining for agglutination at 1:10, 1:100, 1:1000 and 1:10,000 dilutions with physiological saline solution.

After the infusions, mammary glands were palpated for evidence of inflammatory reactions and the milk was also subjected to the modified Whiteside's test. In this test, five drops of the milk was mixed on a glass plate with one drop of four percent sodium hydroxide solution.

RESULTS AND DISCUSSION

Infusion of the Dry Udder

Giltner et al. (4) and Smith et al. (6) have proposed without explanation that antibodies were produced locally in the cow's udder. In this study, the effect of the infusion of an antigen into the udder was studied. Data were collected on the titer of the blood and colostrum at the time of parturition on seven cows whose udders were infused during the dry period with varying amounts of antigen. This revealed that the antibody titer of the colostrum was higher than that of the blood serum at the time of parturition. As lactation advanced, there was a gradual reduction in the antibody content of the milk whereas that in the blood serum remained fairly constant. Similar decline in the milk antibody level has been reported by Mitchell et al. (7) following immunization of the udder with Newcastle's virus and other agents.

Infusion of the Lactating Udder

Additional data were obtained from three cows that were infused with the antigen into one or two quarters of the udder during lactation. The initial response showed the infused quarter to be much higher in antibody content than the blood serum. Antibodies appeared in the uninfused quarters four
days after the initial infusion. These results are in agreement with those obtained by Collidge (8), Giltner et al. (4), Smith et al. (6), and by Mitchell et al. (7). These facts are adjudged by us to be proof of local antibody production in the mammary gland.

The Speed of Response

To determine how quickly the udder will respond to the infusion of antigen, the four quarters of the udder of two lactating cows were each infused with four ml. of the antigen suspended in 16 ml. of distilled water. One hour after the infusion one ml. of oxytocin was administered intravenously so that the contents of the udder could be completely removed. The antibody titer of the milk showed agglutination titer of 1:100 at the end of hour two and a titer of 1:1 in the blood serum at the end of hour 24. Giltner et al. (4) reported that antibodies were detected in milk the day following the infusion of the antigen. Smith et al. (6) reported a response in milk within two days following the infusion. However, in this experiment, a pronounced response in the milk was given two hours after the infusion of the udder, but only a slight response was given in the blood serum 24 hours later. It was clearly demonstrated that the bovine mammary gland is capable of producing large quantities of antibodies, as was evidenced by the response to the infused antigen.

Intramuscular and Subcutaneous Injections

Data were obtained from three cows: one that received one intramuscular injection, and two, each of which received one intramuscular and four subcutaneous injections of varying amounts at seven-day intervals. In comparing the titer of the colostrum and blood serum obtained from these three cows with that obtained from the seven cows infused by the intramammary route, it was apparent that the antibody content of the colostrum was higher from the cows infused through the teat canal.

Booster Shots

To determine if there was a difference in booster response to the antigen administered intramuscularly and by intramammary infusion, eight cows which were already producing antibodies against the antigen were given booster shots by the two different ways. Four cows were administered the antigen via intramuscular route and four received intramammary infusions. In comparing the booster effect of the two methods, it was found that the response obtained from intramammary infusions was within five to nine hours in the milk. Little or no change was noted from the intramuscular injections.

Persistence of Antibody Production

In order to determine the persistence in antibody production, the antibody titer of both the milk and blood serum from four cows that had intramammary infusions while dry was determined at weekly intervals over a period of
246-288 days. It was found that the titer of both the milk and blood serum showed considerable fluctuation, however, the level of both remained high. These findings are in agreement with Mitchell et al. (9) who, using live virus as an antigen, reported that the bovine mammary gland was producing antibodies 18 months after being infused.

Health of Cows Used in this Study
All animals that received intramammary infusions of the antigen while dry showed no marked systemic reaction to the antigen immediately following the infusion or after calving. Typical reactions to intramammary infusions made during lactation were: (1) Elevation in body temperature from two to four degrees, (2) complete letdown of milk in infused quarters within three hours, (3) positive reaction to the Whiteside's test for four days after infusion, and (4) approximately a 50 percent drop in milk production the first day after infusion with a return to normal within five days.

All cows (dry and lactating) that received intramuscular injections of the antigen showed pronounced systemic reactions three to four hours after the injections. Typical of the reactions was an elevation in body temperature of two to four degrees, labored breathing and bloating.

SUMMARY AND CONCLUSIONS
On the basis of these findings, it would appear that there are in the bovine two antibody-producing mechanisms; namely, the mammary gland and the systemic reticuloendothelial system. It would appear that the mammary gland possesses an antibody producing system capable of rapid, copious production of antibodies to specific antigens. This is not unrelated to the reticuloendothelial system of the body for antibodies pass from the gland to the blood stream when the gland is stimulated and similarly antibodies pass from the blood stream to the gland. The stimulation of the gland during the dry period resulted in a higher antibody content in the milk than in the blood serum at time of parturition. However, as lactation advances, the milk drops below the blood in antibody content. Reasons for this may be (1) the constant removal of antibodies from the mammary gland in the milking process, and (2) the reduction of local immunity in the gland.

On the basis of the findings herein reported, the following results are indicated:

1. Intramammary infusions of an antigen made during the dry period resulted in a higher antibody content in the colostrum than in the blood serum.
2. Intramuscular and subcutaneous injections of the antigen made during the dry period resulted in antibody appearance in both colostrum and blood serum but not as much as followed intramammary infusions made during the dry period.
3. Antibodies appeared in the milk within two hours after lactating cows were infused with the antigen.
4. No response in milk antibody levels was obtained from intramuscular booster injections of the antigen, but there was an increase within five to nine hours in the antibody content of the milk from cows after intramammary booster infusions of the antigen had been made.

5. Persistence in antibody content of the milk and blood serum remained at a high level over a period of 246-288 days following immunization during the dry period.

REFERENCES

REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

HOWARD W. JOHNSON, Chairman, Beltsville, Maryland; C. G. BRADT, Ithaca, New York; JOE B. FINLEY, Encinal, Texas; C. A. MANTHEI, Beltsville, Maryland; E. F. KNIPLING, Beltsville, Maryland; W. D. KNOX, Fort Atkinson, Wisconsin; A. W. AGNEW, Milton Junction, Wisconsin; J. G. MILLIGAN, Montgomery, Alabama; J. ARMSTRONG, Salem, Alabama; R. D. TURK, College Station, Texas; L. E. BODENWEISER, Salem, Oregon; H. G. GEYER, Columbus, Ohio; E. M. ELLIS, Kissimmee, Florida.

Mr. Chairman, Members of the Association, and Guests: Your Committee has chosen to give a report on the present situation and research needs of a number of conditions seriously affecting the cattle industry. Your Committee feels that by such a procedure this Association’s attention can best be focused on the disease problems of the industry.

ANAPLASMOSIS

Anaplasmosis is an infectious disease of cattle characterized by anemia and the presence of marginal bodies in the animal’s red blood cells. It is enzootic in the coastal regions of the southeastern and Gulf states and along the Mississippi River valley. It is also enzootic in at least eight of the western states. Two species of wild deer native to California have been shown to be naturally infected with the disease.

The exact nature of the causative agent is not known, although it has been considered to be protozoan-like in many of its properties. The disease agent is readily transmitted by any means whereby blood from an infected animal is rapidly transferred to a susceptible one. Numerous biting insects have been shown capable of such transmission. Several species of ticks have been shown to be capable of transmitting the disease. Evidence of hereditary or transovarian passage of the infectious agent through two species of ticks occurring in the western states has been presented. To date no means for cultivating the etiological agent, other than in the ruminant hosts, has been discovered. The etiological agent usually remains indefinitely in the blood of cattle which have recovered from acute signs of the disease. Diagnosis of the carrier animal is possible by means of testing the animal’s serum with the complement-fixation method. The tetracycline group of antibiotics are inhibitory to the infectious agent within the bovine host. Continuous treatment of carriers with relatively large amounts of these antibiotics will eliminate the carrier infection. However, the animals are again susceptible to the disease, as no immunity follows after the carrier state is lost.

Research progress on anaplasmosis has been slowed by the necessity for using cattle in experimental studies. Expanded efforts to cultivate the etiological agent in tissue culture or in some type of small laboratory animal
would, if successful, provide means for greater research productivity on the disease. The exact nature of the etiological agent needs elucidation and further study. At present there is no laboratory method for detecting whether ticks contain the infective agent or not. Theoretically, certain species of ticks provide the agent with a suitable medium for survival and possibly propagation. However, it has not been possible to determine whether ticks are infected except by feeding them on a susceptible cow or calf. Laboratory studies using ticks could be a very profitable tool if there were means for recognizing the agent in tick tissues. Application of such a method could be used to survey the extent of the disease in naturally occurring ticks. Although ticks have been incriminated as vectors under controlled conditions, their role in the transmission in nature is more speculative than factual. Research studies to determine the true role of insects and ticks in the transmission and perpetuation of the disease are urgently needed. Investigations on methods for the control of vector species found is needed. Further studies on the extent of anaplasmosis in wildlife, such as deer, antelope, and elk, are needed in those areas where the disease is enzootic. The possibilities of such animals playing a reservoir role of the disease, with arthropods being the means of carrying the infection to cattle must be more completely investigated. Research directed towards a means for making cattle resistant to the disease is also needed. Preliminary studies to date on a satisfactory vaccine for anaplasmosis is not encouraging but should be continued.

BOVINE BRUCELLOSIS

The brucellosis eradication program has moved forward with great speed. This has been due mainly to the application of three procedures of proven value. These procedures have been:

1. The seroagglutination test with subsequent slaughter of reactors.
2. The milk ring test as an inexpensive screening device of detecting infected herds.
3. The establishment of a serviceable degree of immunity through calfhood vaccination with *Brucella abortus* strain 19.

However, as the typical cases of bovine brucellosis have been markedly reduced, they have permitted attention to be focused on atypical or unusual cases of the disease. These cases frequently show considerable variation in their etiological, serological and pathological characteristics. At present we have only limited information on their potential epidemiological significance.

Although presently employed procedures have been successful in lowering the incidence of bovine brucellosis they may not be fully adequate for a program of total eradication. The solution to the following problems will facilitate the attainment of our goal.

1. To determine the significance of low level agglutination titers, particularly the differentiation of specific *Brucella* agglutination reactions
from nonspecific reactions and their relationship to persistent post-vaccinal titers.

(2) To obtain specific information on the entire antigenic structure of the Brucella cell so that we may more clearly understand the various antigen-antibody complex reactions, particularly those strains which fail to give positive serological results, yet are capable of producing clinical manifestations of the disease.

(3) To determine the various physical factors and biochemical components that determine virulence and nonvirulence in the Br. abortus cell.

(4) To determine methods for measuring and enhancing the degree of immunity afforded by strain 19 to typical as well as atypical strains of virulent Brucella.

(5) To determine the epidemiological relationships between the three species of Brucella in various hosts, particularly the bovine.

Efforts to achieve the goal of complete eradication should not be relaxed, but instead, pursued with increased vigor!

BOVINE MASTITIS

At present, only mastitis caused by Streptococcus agalactiae can be eradicated from a herd. One reason is that the organism cannot survive for long outside the udder. Because no method of disinfection or milking has been devised which will prevent the spread of mastitis in a herd, regardless of the cause, and because all organisms other than Str. agalactiae are considered ubiquitous in the environment, no attempt is made to eradicate udder infections caused by these organisms. Instead, good management practices, particularly the proper care and use of the milking machine, are advocated as a means of reducing udder injury and thus decrease the incidence of clinical mastitis. There is no doubt that this approach in many instances can do some good, but many of the procedures advocated as doing good are based on circumstantial evidence rather than experimental data. Therefore, more research is needed to define the many management practices which actually influence the spread of mastitis and determine how they act. Our knowledge of the habitats or reservoirs of the many organisms other than Str. agalactiae that cause mastitis is very limited. Until this information is obtained, it will be most difficult to devise any program of eradication of mastitis caused by these organisms. The idea that all of these organisms are ubiquitous is not based on any extensive research data. This is particularly true of hemolytic, coagulase-positive staphylococci. For instance, work at the National Animal Disease Laboratory, now at Beltsville, Maryland, indicates that as long as cows with staphylococcal mastitis are present in a herd, the organism can be readily found on the skin. However, this is not true if all udder infections are eliminated. The fact that a herd of 20 cows has been free of staphylococcal mastitis for several years further indicates that we should not consider it impossible to eradicate from a herd mastitis caused by this organism.
The low incidence of mastitis caused by coliform organisms cannot be attributed to a lack of exposure. Therefore, research is needed to determine if this is due to a high degree of resistance of the cow or to differences in the invasive powers of the strains which cause mastitis and those normally found in the environment. The soft keratin in the teat canal is apparently important in preventing infection but we still do not know how it acts. Nor do we know how such factors as feed and stage of lactation influence the development of clinical mastitis in infected cows and why some cows are more prone to flare-ups than others. These are only a few of the fundamental aspects of mastitis that must be studied before we can hope to have more than stop-gap measures for dealing with mastitis caused by organisms other than \textit{Str. agalactiae}. Data available regarding genetic resistance and predisposition are not conclusive, but warrants further evaluation. The part played by viruses invading the udder needs more investigation.

**BOVINE MUCOSAL DISEASE COMPLEX**

The bovine mucosal disease complex includes those diseases which affect the mucous membranes or tissues that line the digestive and respiratory systems. Some of these diseases are known to be due to specific viral infections, such as rinderpest, malignant catarrhal fever, bluetongue, and infectious rhinotracheitis, as well as another group of conditions most of which are relatively new in the United States and are referred to as New York virus diarrhea, Indiana virus diarrhea, winter dysentery, Iowa mucosal disease, ulcerative stomatitis, and probably other unidentified diseases for which the exact etiological relationship has not been established or proved.

The Animal Disease and Parasite Research Division is supporting research through contracts or cooperative agreements with State agricultural experiment stations at Iowa State University, Ames, Iowa, on Iowa mucosal disease and at Purdue University, Lafayette, Indiana, on the viral diarrhea disease group.

**Iowa Mucosal Disease**

Iowa mucosal disease was first observed in Iowa in 1951 and since then has appeared in all parts of Iowa and all states adjoining Iowa, as well as some other states throughout the country.

The causative agent is unknown and transmission experiments are mostly negative or questionable. However, it is believed that the cause is probably of a viral nature. The clinical and pathological picture is similar to the viral diarrhea diseases, but usually within affected herds there is a very low morbidity incidence averaging two to five percent with practically 100 percent mortality for affected individuals, which is in direct contrast to the viral diarrhea group of diseases. Differential diagnostic tests have shown no relationship of this disease to rinderpest. While transmission trials have been questionable, field observations indicate that there may be a nonclinical form of the disease associated with immunity in most affected herds because recurrences of the disease in affected herds are infrequent.
The etiology must be established and the transmissible agent isolated for experimental studies before any progress can be made towards establishing the relationship of this disease to other viral diseases, as well as developing recommendations for control of the disease.

Bovine Viral Diarrhea Diseases

At the present time there is an absence of knowledge concerning the relationship of viral diarrhea agents which have been isolated, transmitted, and reported in the past as compared with present-day agents because most of the old agents were lost.

The first recognized and reported viral diarrhea agent, identified as New York virus diarrhea, occurred in 1946. Another similar agent was isolated and identified as Indiana virus diarrhea in 1954. Indiana workers reported no immunological cross-immunity between these two viruses. Since both of these original viruses are reported lost, it is not known whether they are the same or different from the present viruses that have been isolated and identified as New York and Indiana viruses and which are reported now by New York workers to be serologically the same. Also, there have been many transmissible viral diarrhea agents isolated by workers in other states, the identification relationship for which is clinically similar but has never been established serologically and immunologically.

The clinical manifestations produced by viral diarrhea agents vary considerably in different locations, as well as in a single area over a period of years. It is not known if these variations are due to different agents or to mutations or changes in a single agent or the immune animal environment. The early New York viral diarrhea disease incidence was reported to be 33 to 88 percent with four to eight percent mortality of affected animals, whereas later in 1955 the morbidity was reported to be less than five percent with 50 percent mortality of affected animals. Indiana reported morbidity incidence of nearly 100 percent with a two-to-four percent mortality.

Different workers have passaged isolated viruses in cattle, rabbits, and tissue culture. Cytopathogenic effects have been observed and serum neutralization tests have been made by some workers. However, it is questionable if present procedures are adequately developed and standardized for controlled use by disease control officials. Drugs or chemicals, such as the antibiotics which would show virucidal activity against the disease, have shown very little value except for treatment of some of the secondary infections. Natural immunity following convalescence, as well as the resistance of nonclinical cases in affected herds to virus challenge, indicates widespread geographic distribution of the disease and suggests the possibility of favorable future developments in the field of immunization.

There is an urgent need for a national central disease agency and specific antiserum repository where agents may be safely stored, passaged and studied to determine the true relationship of agents involved in the viral diarrhea disease complex. The future development of vaccines for control of these diseases would naturally follow the development of knowledge about the
number of diseases, types, strains, and variant viruses for which there were methods of diagnosis and immunization.

BOVINE TUBERCULOSIS

Bovine tuberculosis is causing concern to the livestock sanitary officials because of the increased percentage of reactors and severe outbreaks in herds of cattle previously free of the disease for many years. In addition to the increase in spread of *Mycobacterium tuberculosis* in cattle, half of the animals that react to the tuberculin diagnostic test show no visible lesions on post-mortem inspection. Tuberculin used as the diagnostic test agent, however, is more reliable than the customary post-mortem examination as a means of detecting tuberculosis.

Presently employed procedures of tuberculin testing and slaughter of all reactors have been successful in lowering the incidence of bovine tuberculosis, but they have not been adequate in finding all infected animals. Research is needed to develop more specific diagnostic tests, and to determine the causes of nonspecific reactors. Serological tests need to be explored as a tool to be used in locating infected herds.

BOVINE VibriOSIS

Today, *Vibrio fetus* infection in cattle is widespread in the United States. It causes a high rate, 40 percent, of bovine infertility and thereby is responsible for a tremendous economic loss resulting from decreased reproductive capacity, abortions, decreased milk production, and necessity of replacements.

All females are susceptible to the disease. When the infection first enters a herd, at least 85 percent of the females become repeat breeders. When infection is long standing in a herd, however, previously infected females may become reinfected but exhibit a satisfactory conception rate, whereas susceptible additions experience considerable breeding trouble. Although the period of repeat breeding varies with different females, all recover spontaneously and eventually conceive. Artificial insemination with *V. fetus*-free semen for two calf crops assures recovery.

Approximately 30 percent of the bulls are susceptible to infection.

In addition to *V. fetus*, which is isolated from the reproductive tracts of cattle and aborted fetuses, other Vibrio that are similar in biochemical, serological, and growth characteristics are being isolated from sporadic cases of bovine abortion, reproductive tracts of cattle and sheep, intestinal tracts of cattle, sheep, swine, poultry, and from blood and spinal fluid of man.

For an ultimate disease control program, research is needed for differential identification and determination of pathogenicity of these Vibrios for cattle and other species. Research is also needed for the development of diagnostic techniques, determination of modes of spread, and location of reservoirs of infection.

Artificial insemination has been the most satisfactory method of control and eradication of venereal vibriosis. Education of livestock producers as to methods of sanitation for prevention of infection is necessary.
CURRENT STATUS OF CATTLE INSECT CONTROL

The past 15 years have been a productive period in the development of effective control measures for several damaging insects and ticks that affect cattle. Of equal significance has been the increased interest by the cattlemen in reducing losses that these pests cause by use of recommended control procedures. In past years, the average cattleman did not consider biting flies to be of much importance. Even a moderate infestation of lice and ticks was not always recognized as being deleterious to cattle. With narrowed profit margins and the need to produce more meat and milk economically, the grower recognizes that control of these pests pays dividends.

Before 1945, rotenone pyrethrum and the arsenicals were the primary insecticides for the control of external parasites of cattle. Research since that time has developed excellent insecticide treatments for most of the livestock pests. Sprays of toxaphene, lindane, Co-Ral, ronnel, methoxychlor, and malathion are recommended for louse control. The last two insecticides do not require a holding period between treatment and slaughter, whereas the others require 28 to 56 days in order to meet legal tolerances. These materials are all used as 0.5 percent sprays except lindane which is recommended at 0.03 percent. These insecticides, except methoxychlor, are also recommended for the control of ticks on beef cattle. With the exception of lindane, they are also recommended for control of the horn fly, one of our most costly livestock insects, which may infest an animal by the thousands if not controlled. In addition to conventional sprays, back rubbers impregnated with DDT and toxaphene are useful treatment devices to control this fly.

Significant progress has been made in developing the systemic approach to the control of cattle grubs, pests which cost the livestock industry an estimated loss of $100 million each year. Two materials, ronnel and Co-Ral are now available to livestock growers. Effective cattle grub control can be accomplished with Co-Ral as a 0.5 percent over-all body spray when applied at a rate of one gallon per animal after the active heel fly season. Equally good control can be obtained with ronnel, which is administered as an internal treatment.

In addition to their use as systemics for grub control, Co-Ral and ronnel 0.5 percent sprays are effective as contact insecticides for the protection of animals from screw-worms when applied as an over-all body treatment. These materials destroy larvae in wounds and prevent reinfestation in new and old wounds for about 15 days.

One of the outstanding developments in the field of biology has been the development of a unique method of eradicating the screw-worm by the release of males made sterile by exposure to gamma rays. The practicality of the method was demonstrated by the elimination of the screw-worm from the southeastern states in a large cooperative project between the Florida Livestock Board, other state agencies in the southeast, and the United States Department of Agriculture.
The biting flies such as horse flies, stable flies, and mosquitoes are exceedingly difficult to control on cattle. Synergized pyrethrum sprays are the most effective but do not protect for more than one to three days and are expensive. The development of longer lasting and more effective materials to protect against these flies is urgently needed.

The use of insecticides on lactating dairy animals is limited because of insecticide residues in milk. The only sprays recommended are synergized pyrethrum, the organic thiocyanates, and rotenone for control of lice, horn flies, cattle grubs, horse flies, stable flies, and mosquitoes. Methoxychlor and malathion dusts are approved for horn fly control. Arsenic is still employed by regulatory agencies for the control or eradication of ticks, and diphenylamine and lindane smear preparations for screw-worm infested wounds.

The problem of controlling livestock insects, especially on dairy cows, effectively and economically without leading to residues in excess of rigid tolerances established by regulatory agencies, is one of increasing concern to research workers and the livestock industry.

A strong program of research on all phases of the livestock insect problem to develop more effective and economical treatments is urgently needed. The development of nonresidue-forming insecticides and other ways of controlling cattle insects should be investigated in an expanded program. No effective means has been found for the control of the face fly, a pest and potential carrier of diseases of cattle, which has only recently become established in the United States.

**INFECTIOUS BOVINE RHINOTRACHEITIS**

Infectious bovine rhinotracheitis (red nose, California influenza-like disease) was first reported as a new disease in California dairy cattle in the Los Angeles area in 1953. It was previously observed in Colorado cattle as early as 1950 and continued occurring annually in a few scattered feeder cattle herds until 1955 when a large outbreak occurred involving 250,000 feeder cattle in the northeast quarter of Colorado.

The disease was associated with a high morbidity and low mortality. The Animal Disease and Parasite Research Division established cooperative projects on this disease with state institutions in California and Colorado. Research workers isolated a virus as the causative agent which was successfully adapted to tissue culture from which a successful vaccine was prepared. Serological and immunological tests demonstrated no relationship between this virus and other diseases associated with the mucosal disease complex or shipping fever.

The application of a commercially available vaccine to feeder cattle in previously affected areas has reduced losses to a point where the disease is of little economic importance. Recent research information from Cornell indicates infectious pustular vulvovaginitis and infectious bovine rhinotracheitis of cattle may be caused by the same or closely related viruses. Also, Colorado workers report results from a tissue culture serum neutralization test survey of all Colorado counties which indicate the disease is widely
transmitted in a nonclinical form, as positive reactions were obtained in many breeding herds from all counties including areas where clinical cases had never been observed. The clinical disease apparently results from this nonclinical virus disease when susceptible negatives are mixed with infected animals in association with stress from feed lot environment.

Disease losses are presently being satisfactorily controlled in association with infectious bovine rhinotracheitis modified tissue culture virus vaccination when animals are introduced into feed lots. However, additional information is needed on the carrier cases, virus reservoirs, significance of secondary bacterial infection, method of transmission, and duration of immunity associated with nonclinical virus infections.

JOHNE'S DISEASE

Johne's disease is a chronic infectious disease of ruminants characterized by diarrhea, emaciation, and death. It causes an economic loss because of decreased meat and milk production, necessity of herd replacements, and loss from the sale of breeding stock. The most satisfactory method of control and eradication has been by johnin testing and removing the reactors; also by raising calves on clean grounds away from older animals until they go into the milking line or breeding herd.

The johnin test does not pick up all animals that are infected, especially animals in the latent stages. For an ultimate disease control program, research is needed for diagnosis of the disease in all stages of infection, especially in the early stage when clinical signs are absent. At necropsy, the disease is recognized by the presence of the acid-fast bacillus in the mucous membrane near the ileocecal valve. Methods of concentrating the bacillus, from infected fecal material, in the live animal would result in earlier diagnosis, before the onset of clinical symptoms. Work should be directed also toward the development of satisfactory serological tests which could be used as a herd diagnosis.

LEPTOSPIROSIS

Leptospirosis is a widespread disease not only in this country, but across the world as well. At least 56 serotypes of the organism have been recognized. Eleven of these have been isolated in the United States and serologic evidence suggests the presence of two others. The several serotypes vary in their distribution, host selectivity, and virulence. The wide distribution in wild animal reservoirs, particularly the rodents, makes diagnosis and control an extremely complex problem. Most serological tests are highly specific. So much so, in fact, that a test using one serotype may not detect antibodies against another. Furthermore, antibodies against one serotype may not protect against infection with another serotype.

Much of the present research is directed toward determining the extent of the natural reservoirs and the means of transmission to domestic animals. Once infection is introduced into a herd, the extent and rate of spread as well
as the severity of the disease produced is extremely variable. A thorough understanding of these factors is essential before any logical control program can be formulated.

**SHIPPING FEVER**

Agricultural Research Service veterinarians in 1958 isolated a virus (Myxovirus para-influenza 3, bovine variety) from cattle suffering from shipping fever and have caused signs of shipping fever in susceptible calves by exposing them to this virus. Animals exposed to appropriate Pasteurella strains 24 hours after being exposed to the virus manifested more marked signs of disease than those exposed to the virus alone. Animals exposed to the Pastuerella strains alone manifested only a slight and transient temperature rise.

This evidence of viral-bacterial etiology increases our knowledge of the shipping fever complex, which causes an estimated annual loss of at least 25 million dollars.

The virus of infectious bovine rhinotracheitis has also been isolated from cattle showing signs of shipping fever. In addition the para-influenza 3 virus was isolated from six of 10 herds in which some or all of the cattle exhibited signs of shipping fever. Convalescent sera from cattle in seven of these herds also had at least an eightfold increase in hemagglutination-inhibition antibody titer to this virus.

The para-influenza 3 virus has been isolated from cattle by other workers in California, Kansas, Illinois, Ohio, New York, and Wisconsin as well as in Sweden.

Scientists at the National Institutes of Health, Bethesda, Maryland, have identified this bovine virus and noted its close similarity to para-influenza 3 strains isolated from children with respiratory disease. This is another example of the interrelationship of veterinary and human medicine and of human and animal diseases.

It is now established that viruses are part of the etiological picture in the shipping fever complex. Pasteurella and other bacteria are also found associated with this disease, which appears to be a virus-bacteria stress complex. It is necessary to assess further the relative role of known etiologic agents as well as to search for other agents as yet unknown. There may be chronological as well as geographical variations in etiologic agents. The preliminary findings lead us to believe that the para-influenza 3 virus is a "natural" infection in calves under "normal" herd conditions, and that there are strain differences in the para-influenza viruses isolated from cattle. It may prove possible to prevent serious cases of shipping fever by vaccination against the viral agents involved. But it may be more efficacious to develop immunizing agents against the bacteria involved or to immunize both against viruses and bacteria. All these questions remain to be answered. Their solution will further not only the prevention of losses from shipping fever but the understanding and control of other diseases of complex etiology.
FOREIGN DISEASES

The subject of animal diseases throughout the world is too extensive to discuss in a single report. Our report, therefore, deals only with rinderpest, contagious bovine pleuropneumonia, lumpy skin disease, East Coast fever, malignant catarrhal fever, and trypanosomiasis.

The present knowledge of the distribution and incidence of animal diseases throughout the world was made possible by the Food and Agriculture Organization of the United Nations through the collaboration of many countries. The last world report in 1958 showed that none of the diseases discussed here are now present in the Americas except for some trypanosomiasis in South America and malignant catarrhal fever. These two diseases were reported in three countries.

The report showed an improvement in the rinderpest situation in the Far East. Thailand is now free of the disease. In Africa, lumpy skin disease has extended into new areas and is now regarded as one of the most important cattle diseases in South Africa.

**Rinderpest**

Rinderpest has been successfully eradicated from some areas of the world and is being controlled in others, according to the last world report. The disease has been known since ancient times in Asia, Africa, and Europe. However, all of Europe is now free of the infection. Rinderpest has never been identified in North America, and South America has remained free except for a Brazilian outbreak in 1921. The disease is now confined to part of Asia and Africa where there are still foci of infection.

Since rinderpest is such a deadly ailment, we have given much attention to its diagnosis and control. Recent research has produced more efficient tools for its detection. A modified complement-fixation test for rapid diagnosis has been developed by the Agricultural Research Service. This test is a welcome addition to the recent agar gel precipitin test and the serum-neutralization test.

Available vaccines are of chick embryo, caprinized, and lapinized strains. The caprinized and lapinized strains, particularly the former, are used extensively in Africa. The caprinized strain is effective but may produce severe reactions in many cattle. The degree of reaction is influenced by the innate resistance of susceptibility of different cattle breeds. We need a vaccine that retains the immunological advantage of the caprinized strain but does not cause undue reactions.

Rinderpest is a disease that requires international planning for its control. For such a program, we need additional data on host susceptibility, particularly for swine, and physiochemical studies on the exact chemical structure of the virus components.

**Contagious bovine pleuropneumonia**

Contagious bovine pleuropneumonia (CBPP) has been reported in parts of Asia, Africa, Australia, and Europe. However, no European countries except
Spain reported the disease in 1958. In the nineteenth century, the disease was disseminated to the United States in cattle exported by other countries. Through a program of research and eradication, sponsored by the United States Department of Agriculture, the disease was entirely stamped out by 1892 and has not appeared in this country since.

Vaccination has been widely used in CBPP control, but it has not been successful in eradication. The use of attenuated culture vaccines has been moderately successful; however, these vaccines do not have the precise attenuation for optimal immune response and minimal reaction. Research has produced much data and valid methods for diagnostic use, and serological tests can now confirm the clinical history and histopathological studies.

**Lumpy skin disease**

Epizootics of lumpy skin disease have been reported from Africa, and the infection has spread to new areas in Asia, namely, Ceylon and North Borneo. No cases were reported from Europe in 1958, although the disease had previously been reported from Yugoslavia. The continents of North and South America are free of the disease.

The infection first appeared in Africa and has spread widely from the regions where it was first found. The disease first appeared in northern Rhodesia and was described in 1931 as “pseudo-urticaria.” About 14 years later, the disease appeared in the Transvaal; the first experimental transmission was accomplished in 1945 at Onderstepoort. The epizootic pattern was unique, since the disease spread in an obscure manner and recurred at widely separated points without known contact between infected and susceptible animals.

Diagnosis is made on the basis of clinical observations, histological examinations, and the epizootic pattern. The disease should be differentiated from ephemeral fever (three-day sickness), foot-and-mouth disease, photosensitization, urticaria, Demodex infection, and insect bites. Current studies are being carried out in Africa to develop accurate diagnostic tools, such as serological tests, the agar gel precipitin test, and tissue culture of the virus. However, diagnosis and perhaps control are further complicated by the existence of at least three types of viral agents associated with the disease. Quarantine enforcement and attempts to control insects have not prevented its spread.

A vaccine prepared from the developing chick embryo was used extensively in 1955, but the results were not encouraging. However, we recently found that a vaccine prepared from sheep pox virus would protect cattle. According to unpublished reports, this vaccine is currently used without undue reaction.

This cross-protection is another example of the relationships among some disease agents that are apparently unrelated in clinical manifestations. We need further serological and biochemico-biological studies to understand why heterologous viruses sometimes produce homologous reactions in immunization.
East Coast fever

East Coast fever is a highly fatal disease of cattle, which occurs enzootically along the coastal regions of equatorial East Africa. From the enzootic regions, it has spread to other areas. The last world report showed the disease in seven African countries. It is not known to exist outside of Africa at this time although two Malayan countries—Sarawak and Indonesia—did report the disease in 1956 but not in 1958.

This infection, which is tick-transmitted, is attributed to a protozoan organism, *Theileria parva*. However, we still lack valid criteria for a clear-cut classification of this group of protozoans. In 1956, workers proposed at Onderstepoort that *Theileria parva* be reclassified on the basis of its life cycle. Such a reclassification might help in clarifying this group of protozoans, particularly as to diagnosis and transmission of infection. We need to understand why the virulence varies within a given species. The present diagnostic procedures do permit differentiation, but they are time consuming and costly. A definitive differential diagnosis depends on cross-immunity tests.

The possible usefulness of serological tests has not yet been fully explored. Control methods—such as slaughter of infected and exposed animals, strict quarantine, and eradication of the tick vector—have not been successful. Despite these methods, the disease continues to exist.

Malignant catarrhal fever

This disease seems to occur in cattle-raising areas in all parts of the world. In the American continents, three out of 24 countries had infected cattle, according to the last world report. The United States and Columbia reported sporadic cases. The disease was suspected, but not confirmed, in Peru.

Studies indicate that some game animals and sheep may be reservoir hosts. Contact between domestic cattle and wild animals, particularly deer and the African wildebeeste, should be prevented. Recovered cattle seem to have some immunity; therefore, hyperimmunization has not been successful, and observations on immune response have discouraged attempts to develop reliable immunizing agents. Experimentally, an immunity has been demonstrated that lasted four to six months, but heterologous virus types did not give cross-protection.

Histopathological examination, particularly for perivascular infiltration, is satisfactory for differential diagnosis if coupled with a suggestive history, especially in endemic areas. In such areas, a history of contact with wild game is highly significant. The definite mode of transmission is unknown, and the disease may be confused with a composite of "mucosal diseases." Therefore, more precise diagnostic methods are needed before comparative studies can be made of cases in widely separated areas.

Trypanosomiasis

Trypanosomiasis, also called "nagana" and tsetse fly disease, is caused by trypanosomes that are transmitted by various species of tsetse flies (genus
Glossina). It has been found in most areas of Africa where tsetse flies are present. In the last world survey, 20 of 35 African countries reporting the disease had infection from both Trypanosoma congolense and T. vivax. The disease was widespread in 10 African countries and present only in imported animals in one country. It was described as reduced, confined, or sporadic in 24 countries. Three South American countries also reported T. vivax infection but not T. congolense. Man as well as domestic animals may be susceptible to trypanosomes carried by the tsetse fly.

Prevention of trypanosomiasis among livestock depends on elimination of the tsetse fly vector, although a number of drugs are now available as a prophylaxis and cure for the disease in livestock. Ten species of Glossina are known vectors. None of these occur in the United States, but other arthropods in this country have been incriminated as mechanical vectors of pathogenic trypanosomes.

Confirmatory diagnosis can be made by microscopic examination of blood smears. Serological tests have limited value, and further research is needed to develop tests for differential diagnosis.

Recently, two species of tsetse fly, G. swynnertoni and G. morsitans, were found feeding on the African wart hog. The G. morsitans will also feed on the hippopotamus; therefore, elimination of the wart hog would not deprive the vector of his only feeding source. In Zanzibar, the G. austeni feeds only on bush pigs and domestic cattle, hence, destruction of the bush pigs might greatly aid control of the disease.

The land use program now in tsetse fly areas aims to reduce, although not necessarily eliminate, the insect menace. Insect control can be accomplished, but methods now employed are still costly and will limit spread of the infection if combined with treatment by a trypanocidal agent and good management. Increased interest in drug therapy has produced encouraging data. Two drugs, Prothideum and Berenil, are reported to have protective and therapeutic value, respectively.
THE USE OF ORGANIC PHOSPHORUS COMPOUNDS IN DESTROYING OESTRUS OVIS LARVAE


Albuquerque, New Mexico

The nasal botfly, Oestrus ovis, is one of the commonest parasites of sheep in New Mexico. Our work on its control shows that approximately 95 percent of the sheep are infested with this parasite at some time during their lives.

The compounds mentioned in this report, the manufacturers, and the chemical definitions are:

CO-RAL (Bayer 21/199), Chemagro Corp., Kansas City, Mo., O,S-(3-chloro-4-methylumbelliferone) O,S-diethyl phosphorothioate;

Dowco 109, Dow Chemical Co., Midland, Mich., O-(4-tert-butyl-2-chlorophenyl) O-methyl methylphosphoramidothioate;

Ruelene, Dow Chemical Co., Midland, Mich., 4-tert-butyl-2-chlorophenyl 0-methyl methylphosphoramidate;

Dimethoate, American Cyanamid Co., Pearl River, New York, O,S-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate;

Ronnel (Dow ET-57), Dow Chemical Company, Midland, Mich., O,S-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate, and also marketed under the name of Trolene for oral use and Korlan for spray use.

The adult fly is active in the vicinity of Albuquerque, New Mexico, from April until the first killing frost, which is generally in October (1). The female deposits larvae on the nostrils and upper lips of sheep, and these larvae quickly migrate into the nasal cavity. The first instars are usually found in the mucus of the membranes of the nasal septum, nasal meatuses, and turbinate bones. The youngest first instars in the nasal cavity are about one mm., but they attain a length of four or five mm. as they grow. When a larva develops two terminal spiracles, it has reached the second instar stage. During this stage, larvae are usually found in the deepest recesses of the nasal cavity, often between the processes of the ethmoid turbinates or in the frontal sinuses. At this stage, they are five to 13 mm. long. The third or final instars are found in the frontal sinuses, although they may pass through the nasal cavity while emerging from the host animal. The fully grown larvae drop from the nostrils of the sheep, burrow into the ground, and pupate. The pupation period may vary considerably, depending upon the climatic conditions. The average pupation period in the laboratory is about 38 days. Under favorable natural conditions, the time may be considerably shorter; under unfavorable climatic conditions, it is doubtless longer.

* Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Albuquerque, New Mexico.

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An examination of several hundred New Mexico sheep at different times of the year indicates that there are at least two generations of flies annually in this area. The first generation emerges from its puparium in early spring. After fly strike, the earliest larvae can complete their development through the third instar by late summer and pupate as flies in late August and early September. During this time, the sheep are reinfested; an examination of infested sheep during October, November and December shows that they are primarily infested with first instars. Since the first instar is the stage most easily destroyed with parasiticides, the optimal treatment time is following the first killing frost in the fall and during November and December.

It was reported in 1958 that a single oral treatment of Trolene, 100 mg./kg. of body weight, effectively destroyed first instars of *O. ovis* in the nasal cavities of sheep (2). This work was followed in 1959 by a report on the effectiveness of a single intramuscular treatment of dimethoate for destroying first, second, and third instars in the nasal cavities and frontal sinuses of infested sheep (3). During 1958 and 1959, similar tests were made with CO-RAL, Dowco 109, Ruelene, and dimethoate. The latter was used 10 percent premixed as a feed additive.

**MATERIALS AND METHODS**

The sheep used in these tests were naturally infested and originated from ranges and irrigated pastures in New Mexico and Colorado. Most of them were from public sales yards and ranches. Others were from our laboratory flock at Albuquerque, New Mexico. Those from the sales yards were generally cull sheep in very poor condition, but those from ranches and the laboratory were in good condition.

CO-RAL was used as a dip at 0.5 percent concentration prepared from 25 percent wettable powder. In preliminary toxicity tests, Dowco 109 was used on three sheep at 300 mg./kg. In the tests reported here, Dowco 109 and Ruelene were administered orally as boluses, and 25 percent wettable powder in capsules with a balling gun. Dowco 109 was used at dosages of 200, 100, 75, and 50 mg./kg. of body weight. Ruelene was used at dosages of 200, 150, and 75 mg./kg. of body weight.

Dimethoate, premixed 10 percent in cottonseed meal, was fed as a feed additive in one-half pound of ground alfalfa and grain to seven sheep. Individual portions were prepared and fed daily in individual feed boxes to sheep in separate stanchions. The sheep were left in the stanchions until all the feed was eaten. The daily dosage was five mg./kg. for five days. This test was duplicated, using the dosage in capsule instead of in feed.

The sheep treated with CO-RAL, Dowco 109, and Ruelene were killed and examined for larvae 72 hours after treatment. Those given the premixed Dimethoate as a feed additive and in capsule, were killed and examined five days after the end of the treatment period.

The head of each sheep was removed, skinned, and divided sagitally with a saw-cut immediately to the right of the nasal septum, thereby exposing the
ethmoid processes, turbinates, and meatuses of the right nasal cavity. The septum was then removed, exposing the corresponding structures of the left side. To complete the examination of all structures, it was necessary to unfold the turbinates. Each frontal sinus was opened by removing the external surface of the nasal frontal bones by a single saw-cut starting near the supra-orbital crest and ending in the median plane.

All larvae in the treated and untreated animals were counted and classified as to location and larval stage. Although the evaluation was determined entirely by comparing the numbers of live larvae found in treated and untreated sheep, the dead larvae were also counted. The dead larvae were classified as either killed or not killed by the treatment. If they were decomposed, disintegrated, and calcified or if they contained pus, they were presumed to have died before treatment was started. If the dead larvae showed none of these characteristics and were turgid or possessed some resiliency, they were classified as killed by the treatment. Relatively few dead larvae were found, and probably most of them were swallowed after treatment. Therefore, the evaluation of the treatment is based on the number of living larvae found in the treated and untreated groups.

RESULTS

CO-RAL as a 0.5 percent dip, prepared from 25 percent wettable powder, destroyed 97 percent of the first instars in seven sheep. No effect was observed upon the second or third instars. Signs of toxicity were not observed in any treated sheep. (Table I)

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<th>Animal Number</th>
<th>Date Treated: 8/11/58</th>
<th>Date Examined: 8/14/58</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Infestation</td>
<td>Live Instars</td>
</tr>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>B-2956</td>
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<td>0</td>
</tr>
<tr>
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<td>3</td>
</tr>
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<tr>
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<tr>
<td>Percent Control</td>
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<td>47</td>
</tr>
</tbody>
</table>

* Very poor physical condition.

Dowco 109, given to 33 sheep orally in capsules as 25 percent wettable powder, was 100 percent effective at dosage levels of 200, 100, and 75 mg./kg. At 50 mg./kg., it was 100 percent effective against first and second instars and 90 percent effective against third instars. Toxic signs were not observed at these levels. (Tables II-VII)
### TABLE II

The Effect of Dowco 109 on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Weight Kg.</th>
<th>1st Infestation</th>
<th>2nd Infestation</th>
<th>3rd Infestation</th>
<th>Date Treated: 7/1/58</th>
<th>Date Examined: 7/10/58</th>
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<td>2886‡</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>12</td>
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<tr>
<td>Average</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>12</td>
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<tr>
<td>Percent Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Toxic.
† 50% EC in capsules.
‡ Bolus.

### TABLE III

The Effect of Dowco 109 on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Weight Kg.</th>
<th>1st Infestation</th>
<th>2nd Infestation</th>
<th>3rd Infestation</th>
<th>Date Treated: 8/20/58</th>
<th>Date Examined: 8/29/58</th>
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<tr>
<td>B-2951</td>
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</table>

### TABLE IV

The Effect of Dowco 109 on *Oestrus Ovis* Larvae

<table>
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<th>Animal Number</th>
<th>Weight Kg.</th>
<th>1st Infestation</th>
<th>2nd Infestation</th>
<th>3rd Infestation</th>
<th>Date Treated: 1/30/59</th>
<th>Date Examined: 2/2/59</th>
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<td>B-7443</td>
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<td>B-7445</td>
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</table>
TABLE V
The Effect of Dowco 109 on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (Kgs.)</th>
<th>Principals Infestation 1st</th>
<th>Live Instars 2nd</th>
<th>3rd</th>
<th>Animal</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
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<tr>
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TABLE VI
The Effect of Dowco 109 on *Oestrus Ovis* Larvae

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<tr>
<th>Animal</th>
<th>Weight (Kgs.)</th>
<th>Principals Infestation 1st</th>
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TABLE VII
The Effect of Dowco 109 on *Oestrus Ovis* Larvae

<table>
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<th>Weight (Kgs.)</th>
<th>Principals Infestation 1st</th>
<th>Live Instars 2nd</th>
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<tr>
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<td>100</td>
<td>100</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
USE OF PHOSPHORUS IN DESTROYING OESTRUS OVIS 183

Ruelene, administered to four sheep as a drench prepared from 25 percent wettable powder at the rate of 200 mg./kg. was 100 percent effective against first instars, 65 percent against seconds, and 95.0 percent against third instars. When administered to five sheep orally in capsules at the same dosage, it was almost as effective—98.5 percent against first instars and 97 percent against seconds and thirds. Ruelene given to seven sheep as 25 percent wettable powder in capsules at a dosage level of 150 mg./kg. was 100 percent effective against first instars, 75 percent against seconds, and 94 percent against thirds. The percentage of control obtained in seven sheep with 75 mg./kg. was 96 percent against the first instars, 61.5 percent against the seconds, and 82 percent against the thirds. (Tables VIII-XI)

### TABLE VIII

The Effect of Ruelene on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Animal Weight</th>
<th>Infestation Live Instars</th>
<th>Date Treated: 4/20/59</th>
<th>Date Examined: 4/23/59</th>
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</thead>
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<tr>
<td>Percent Control</td>
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<td>95.0</td>
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</table>

* Toxic symptoms observed.

### TABLE IX

The Effect of Ruelene on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Animal Weight</th>
<th>Infestation Live Instars</th>
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<th>Date Examined: 4/23/59</th>
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<td>7469</td>
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<td>0</td>
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<tr>
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<td>Percent Control</td>
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<td>97.0</td>
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TABLE X

The Effect of Ruelene on *Oestrus Ovis* Larvae

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<th>Infestation (Live Instars)</th>
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<th>3rd</th>
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<td>4</td>
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</tr>
</tbody>
</table>

Totals: 0 14 7
Average: 0 2 1
Percent Control: 100 75 94

TABLE XI

The Effect of Ruelene on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Weight (Kgs)</th>
<th>Infestation (Live Instars)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
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</tbody>
</table>

Totals: 6 21 21
Average: 0.85 3.0 3.0
Percent Control: 96 61.5 82.0

Dimethoate, 10 percent premixed, added to feed was nearly 100 percent effective against first and second instars and 98 percent effective against the thirds in one test with seven sheep. In another test using the same dosage in capsule and the same number of sheep, it was 100 percent effective against the first instars, 88 percent against the seconds, and 100 percent against the thirds. No toxic signs were observed. (Tables XII & XIII)


TABLE XII
The Effect of Dimethoate (Feed Additive) on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Weight Kgs.</th>
<th>Infestation Live Instars</th>
<th>Controls Infestation Live Instars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
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<tr>
<td>Percent Control</td>
<td>100 100 98.0</td>
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</table>

TABLE XIII
The Effect of Dimethoate (Capsule) on *Oestrus Ovis* Larvae

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Weight Kgs.</th>
<th>Infestation Live Instars</th>
<th>Controls Infestation Live Instars</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
<tr>
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<td>7471 27 3 6</td>
</tr>
<tr>
<td>7472</td>
<td>35.8</td>
<td>0 0 0</td>
<td>7476 14 12 10</td>
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<td>0 0 0</td>
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<tr>
<td>7484</td>
<td>39.0</td>
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<td></td>
</tr>
<tr>
<td>Totals</td>
<td>0 5 0</td>
<td>134 31 47</td>
<td>Average 0 0.7 0</td>
</tr>
<tr>
<td>Percent Control</td>
<td>100 88 100</td>
<td></td>
<td></td>
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DISCUSSION

The results showed a high degree of effectiveness against the infective larvae of *O. ovis*. Of particular interest are the limited data on the effectiveness of CO-RAL, 25 percent wettable powder, used as a 0.5 percent dip against the first instars. Since only seven sheep were treated, additional information and treatment under field conditions are needed to prove the effectiveness and, above all, the safety of 0.5 percent CO-RAL dip.

Dowco 109, administered orally, was highly effective without toxicity at dosage levels of 200, 100, 75, and 50 mg./kg. of body weight. These data,
therefore, show that a relatively wide safety margin for sheep exists with oral use of this compound against *O. ovis* infection.

Ruelene, given orally, was not as effective as Dowco 109. These data indicate that 200 mg./kg. of body weight of Ruelene were required to produce results comparable to those observed with 50 mg./kg. of body weight of Dowco 109 (Tables VIII & VII respectively).

Dimethoate, 10 percent premixed, given orally as a feed additive at the daily rate of five mg./kg. for five days, was nearly 100 percent effective against all stages of this parasite. Although there was some rejection of the feed by the test animals, such treatment would seem to be practical for parasite control if the feed could be made palatable. The same dosage in capsule produced similarly high effectiveness with no toxic reaction.

Since most of these compounds are still being used experimentally and the data presented here are quite limited, no specific recommendations can be made. Because the compounds appear to be highly effective, however, additional field trials are indicated in areas where *O. ovis* control is desirable.

**SUMMARY**

Controlled experiments were made using organic phosphorus compounds against the larvae of the nasal botfly of sheep. The following compounds were used: CO-RAL as a 0.5 percent aqueous suspension dip; Dowco 109 and Ruelene administered in boluses and in wettable powder form in capsules; and dimethoate as a 10 percent premixed formulation.

As used in these experiments, the compounds were safe and effective in destroying *Oestrus ovis* larvae. The only toxicity encountered was with use of Dowco 109 at 300 mg./kg.

**REFERENCES**


REPORT OF COMMITTEE ON PARASITIC DISEASES

V. D. CHADWICK, Chairman, Jackson, Mississippi; R. L. CUFF, Kansas City, Missouri; F. D. ENZIE, Beltsville, Maryland; J. L. HOURRIGAN, Arlington, Virginia; A. E. JANAWICZ, Montpelier, Vermont; F. R. KOUTZ, Columbus, Ohio; F. L. SCHNEIDER, Albuquerque, New Mexico; L. E. SWANSON, Gainesville, Florida; W. C. TOBIN, Denver, Colorado; R. D. TURK, College Station, Texas.

STATUS OF SCREWWORM ERADICATION PROGRAM

No screwworm infestations arising from the southeastern overwintering area have been found since June 1959. State and federal field employees have continued survey operations and systematic animal inspections for the purpose of detecting the presence of screwworms throughout the former fly release area in Florida, Georgia, and Alabama.

Efforts to protect the entire southeast eradication area from reinfection have included continuous operation of 13 inspection stations along the eastern boundaries of Arkansas and Louisiana. Inspectors at the stations have prevented the entrance of 21 screwworm infestations into the southeast during the first nine months of 1960. All livestock entering the southeast from the southwest must be unloaded and inspected at one of the stations. All wounds on the livestock are treated with an approved smear, and an organic phosphorus insecticide is applied to all animals, with certain exceptions, as a precautionary measure.

Additional inspection is provided seasonally to prevent infested animals from entering the eradication area from the north and midwest during warm months when screwworms may become established in those areas. Air, rail, and ocean shipments of livestock are also certified free from screwworms before entering the eradication area.

The first infestation of screwworms in Mississippi in 1960 was discovered in native animals in the northwestern part of the state in late July. The location of this infestation followed a pattern noted during the previous two years. An investigation is underway, aimed at learning reasons for the consistency of this pattern of spread.

Screwworm data collection was begun early in 1960 in Texas for the purpose of learning more about overwintering habits and seasonal spread of the parasite. Approximately 10 rancher-cooperators in each Texas county were asked to collect larvae from their infested animals and mail them to state-federal laboratories for positive identification.

Cooperation in this effort has been excellent. During the period January through mid-September, ranchers in all but 30 of the 254 Texas counties had submitted one or more specimens that were positively identified as screwworms.
On August 30, a single infestation of screwworms was collected by a local veterinary practitioner from a wounded steer at a Geneva County, Alabama, stockyards. The animal had been shipped from Texas to Alabama a few days prior to the discovery. This represented the easternmost infestation reported since mid-June 1959, when screwworms were last found in Highlands County, Florida.

Precautionary measures taken in connection with the Alabama infestation included cooperative federal-state inspection of all livestock within a 25-mile radius of the point of discovery. Nearby growers cooperated by notifying authorities when they planned to ship livestock. Animals in the stockyards at the time of the discovery were inspected and found free from screwworms. The truck used for transporting the infested animals was thoroughly cleaned and treated with an insecticide.

In addition to these precautions, animals with wounds susceptible to screwworm attack and located in the vicinity of the control pen, were frequently inspected for evidence of infestation with negative results.

CATTLE FEVER TICK ERADICATION

On May 31, 1960, cattle fever ticks (Boophilus microplus) were found at the Okeechobee Livestock Market at Okeechobee, Florida, by a state inspector. The lot of cattle in which the infestation was found had been trucked to the market from Palm Beach County. Additional infested cattle were found in the herd of origin.

A triangular area of approximately 600 square miles involving parts of Palm Beach and Martin counties was placed under state quarantine and all herds within the area inspected.

On June 20, a cattle fever tick was found by a state livestock inspector on a cow at the Cattlemen’s Livestock Market, Tampa, Hillsborough County, Florida. Effective July 1, 1960, Part 72, 9 CFR was amended to place a federal quarantine on Martin, Palm Beach, and Hillsborough counties; and on August 22 on parts of Indian River and Osceola counties. All infested and directly exposed herds were placed under State quarantine. Additional state and federal inspectors were assigned to tick eradication activities and a vigorous eradication program was instituted. Sixteen infested herds in the following counties have been reported: Palm Beach 10, Martin four, Hillsborough one, and Indian River one.

The eradication program includes the establishment of a State Tick Quarantine Line crossing Florida at Ocala (the tick quarantine line is located at the former screwworm quarantine line established in May 1958 and discontinued in November, 1959). Florida regulations require inspection and dipping of animals moving north across the quarantine line. Systematic inspection of all cattle and horses south of the State Tick Quarantine is planned.

Heavy rains have slowed operations and some owners spray their cattle making it more difficult to find ticks.

The program also includes inspection of cattle at slaughterhouses and inspection and dipping of cattle at auction markets.
Previously, on April 23, 1957, cattle fever ticks (*Boophilus microplus*) were found at the Okeechobee Livestock Market; and during the summer of 1957 eight infested herds were found in Okeechobee County, three in Broward, two in Highlands, and one each in Dade and Palm Beach counties. The campaign to eradicate the ticks was pronounced successful in September, 1958, more than a year following the last known infestation.

A previous outbreak of cattle fever ticks occurred in Okeechobee County in the winter of 1945, when quarantines were placed on that county as well as on Glades and Highlands and parts of Osceola and Polk counties.

During fiscal year 1947, infestations were reported in Palm Beach, Broward, Charlotte, Collier, Dade, Hendry, and Lee counties and during fiscal year 1948 in Volusia, Putnam, Flagler, Brevard, Osceola, Lake St. Johns, Alachua, Orange, Madison, and Jackson counties in Florida and on one premises in Brantley County, Georgia. Movements of cattle from Volusia County caused widespread dissemination of the ticks. Systematic inspections and dippings brought the outbreak under control, and the remaining federal quarantines were removed in December 1950.

Florida was the last state, with the exception of Texas where a narrow buffer zone along the international border is still under federal and state quarantine, to be freed of fever ticks. The remaining federal quarantine in Collier and Hendry counties in the Big Cypress Swamp was lifted in December, 1943.

During fiscal year 1960, the following activities were reported in the buffer area in Texas: 91 Mexican livestock and 31 native American livestock straying into Mexico and returning were caught by tick inspectors. Of the Mexican livestock, 41 were horses of which three were found to be tick infested, and 50 were cattle of which 31 were infested. Also animals in four United States herds were found to be infested, and 21 herds were being held for further treatment. Last year 12,435 certificates were issued covering the intrastate movement of 76,659 livestock from the area. In addition 172 certificates were issued for the interstate movement of 21,390 livestock. Also 48,151 herds of 1,045,876 livestock were inspected for ticks, and 9,938 herds of 53,790 livestock were dipped.

Ticks identified as *Rhipicephalus evertsi* were found in September, 1960 at Africa, U. S. A., an animal farm located in Palm Beach County, Florida. This tick is known in Africa to be a vector of East Coast fever and other exotic diseases. It is also a vector of bovine piroplasmosis. This tick had not previously been reported in the United States.

Your Committee recalls that in 1956, other exotic ticks, *Amblyomma gemma* and *Hyalomma* sp., probably *Hyalomma aegyptium*, were found on African rhinoceroses at the Dallas Zoo in Texas.

Your Committee urges that an aggressive tick eradication program be followed in Florida in order that all possible foci of tick infestation be located and eliminated by closely adhering to standard tick eradication procedures. We also urge that import requirements be strengthened to prevent the importation of exotic ticks or other parasites.
SHEEP AND CATTLE SCABIES ERADICATION

Your Committee in reviewing the cattle scabies situation observes that there were four outbreaks during last fiscal year including one each in Oregon, Colorado, Iowa, and Indiana. The one infected animal in the latter state, in a dairy herd, was found by a regulatory veterinarian conducting the tuberculin test. The other three outbreaks were reported by veterinary practitioners. This points out the necessity for working closely with them as well as persons associated with feedlots and auction markets and increased alertness by regulatory inspectors in order to locate any foci of the disease.

Your Committee also reviewed reports of sheep scabies during the past five years and was disturbed over the marked increase in incidence of this disease. In 1955, 24 states reported 442 infected flocks in 219 counties. At public stockyards, 72 infected lots were found. In 1960, the latter had increased to 214 infected lots and 25 states reported 886 infected flocks in 280 counties. The vast majority of the infected flocks were reported in midwestern states; however, two western states—Colorado and New Mexico—also suffered outbreaks. In the latter state, two outbreaks in March and in July were disclosed at an Albuquerque packing plant. Considerable effort was made to locate all foci of the disease but no additional infected sheep were found. In Colorado the disease had been introduced by importation from an infected state.

In order to give better protection to the free area and to give impetus to sheep scabies eradication, Part 74, Title 9 CFR was amended August 1. 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.

Eastern South Dakota was the first eradication area. The states of Arkansas and Tennessee are preparing to qualify and other infected states have expressed interest.

The regulation provides for the official inspection and dipping of infected and exposed sheep prior to interstate movement. The regulation also requires inspection and dipping of all sheep from the infected area moving to the free or eradication areas, except movements to recognized stockyards and slaughtering establishments. Sheep from the free areas may move without restriction. The movement of clean sheep between states in the infected area is not controlled by the regulation.

During the year psoroptic mites were found on elk in Idaho and Wyoming. Psoroptic mites have been found over a period of many years on elk and on big horn sheep in western states. The presence of scabies in wildlife suggests the possibility that they may be a reservoir from which the disease may spread to domestic animals. There is no good evidence, at least during recent years, that this has occurred. Two requirements are necessary in order for this to happen: First, mites on wild life must be able to propagate on domestic animals and secondly, there must be the opportunity for them to spread from wild to domestic animals. Outbreaks during recent years in cattle and sheep are not believed to have been caused in this manner.
PARASITIC DISEASES

It is a problem that deserves further study.

A survey of goat herds in Texas disclosed that many goats over a wide area in the state are infested with psoroptic ear mites. It is not presently known that this parasitic infestation is of economic significance to the goat industry or that these animals are a possible reservoir of infection for sheep and cattle. Research is being conducted to determine whether this parasite is confined to the goat or can be transmitted by goats to sheep thus producing sheep scabies.

Tests with toxaphene for the control of psoroptic scabies in sheep have proved that this compound is a very effective acaricide. Experiments conducted in Illinois and New Mexico with heavily infested sheep have shown that the animals remained free of mites during an inspection period of 17 months after a single dipping in 0.5 percent toxaphene prepared from an emulsifiable concentrate.

Controlled tests under laboratory conditions with CO-RAL, malathion, Korlan, Tedion, Bayer 28589, Bayer 30686, and Dylox, have shown that none of these compounds, with the possible exception of CO-RAL, are as suitable as the presently recommended compounds, lindane or toxaphene, for the control of sheep scabies.

Your Committee is pleased to note that there is now a great deal of interest in completely eradicating sheep scabies from the United States and urges that the eradication program be stepped up in order that this can be accomplished. We also urge that concentrated efforts be directed toward developing better acaricides, particularly those that do not involve a residue problem.

PHENOTHIAZINE RESISTANCE

In the past few years, there have been increasing numbers of field reports suggesting that phenothiazine is less effective than formerly in controlling nematode parasites of ruminants. These reports have come from different geographical areas in this country as well as from abroad (i.e., Australia), and not infrequently the field failures have been ascribed to drug resistance. Until Drudge and his co-workers (1957) published their findings in sheep, however, there was no supportive experimental evidence of such phenomenon. Indeed, thorough investigation usually revealed reasons other than drug tolerance (inadequate dosage, improper preparation and use of free-choice mixtures, overstocking of pastures, inadequate surveillance of herds or flocks, unwarranted reliance on the chemical to control all helminth species, and use of substandard commercial phenothiazine preparations) for the apparent failures of the chemical in the field.

Preliminary studies at Beltsville with several strains of the common stomach worm, Haemonchus contortus, of sheep (the only species with which phenothiazine resistance has been associated) confirmed earlier findings in Kentucky of quantitative phenothiazine resistance in one (strain B, Drudge et al., 1957 et seq.) and revealed resistance of an apparently comparable degree in a strain isolated from animals in one of the local flocks. It is particularly
noteworthy, however, that a statistically significant degree of resistance was not demonstrable in a strain obtained from another local flock nor in a strain from New York State which was reported to be unresponsive to conventional doses of N.F. grades of the drug.

A consideration of available evidence indicates that relative resistance to phenothiazine may occur in certain strains of *Haemonchus contortus* in sheep but that many, and perhaps most, apparent failures of the chemical are not, and should not be, ascribable to this phenomenon. It is emphasized that resistance has been associated only with certain strains of a single helminth species and that sufficient evidence has not been obtained to justify elimination of the drug from all parasite control programs. Every instance of apparent failure of the drug should be investigated thoroughly and judgment rendered in accordance with the specific findings. Alternative treatments (copper sulfate solution, "cunic") may then be advisable when reasons other than apparent drug tolerance have been eliminated.

**CATTLE GRUBS: Hypoderma lineatum and Hypoderma bovis**

The most promising systemic insecticides for the control of cattle grubs are CQ-RIIL and Ruelene. CQ-RAL is usually employed as an aqueous spray applied under high pressure, or by means of the Spray-Dip machine. Ruelene is being used with increasing frequency as a spray prepared from emulsifiable concentrates. Spraying cattle with high powered and Spray-Dip machines, however, is very expensive. A new approach to the problem of grub control consists in applying systemic insecticides in high concentrations to only a part of the body. For example, 250 cc of a 2.0 percent CQ-RAL wash, applied to the backs only, may be a more economical method of treating cattle for grubs than complete coverage with 0.5 percent sprays applied with power equipment or Spray-Dip machine.

**SHEEP KED: Melophagus ovinus**

Large scale tests made by United States Department of Agriculture with 1.5 percent dieldrin dusts for the control of sheep keds in New Mexico for the past three years have shown that the procedure is a satisfactory and economical method for controlling this parasite. The dust was applied with a commercial dusting machine set up at the end of a chute. The sheep are run through a thin cloud of dieldrin dust, and sufficient insecticide penetrates the wool to bring about a very high degree of control. Eradication is not achieved because the dust formulations so far used do not kill the pupae, nor do they have sufficient residual effect to kill all the keds hatching from the pupae. The best time to treat sheep is at shearing time, or as soon thereafter as possible.

**RECOMMENDATIONS**

Your Committee recommends:

1. *Sheep Scabies Control*—As at least one half of all states still have sheep infected with scabies and as one thorough treatment in approved dips, in
most cases, rids sheep of scabies, keds and lice, in addition to protecting
dipped sheep from flies for a few weeks, and so many sheep and lambs are
sold through federal and state supervised markets, and community sales, we
recommend that all sheep for breeding and feeding purposes be dipped under
official supervision before leaving all markets, and all sales.

2. Cattle Grub Control—As the annual cattle grub tax to the industry,
mostly to growers, amounts to at least $100,000,000 and as the three federally
approved systemics, CO-RAL as a spray; Trolene, as a bolus; and Ronnel
granules, to be mixed in feed, control cattle grubs, when used as directed, in
addition to controlling lice, ticks, some flies, and also to act as an anthelmetic
on some intestinal parasites. We recommend the proper use of these approved
systemics. It is reported that exceptionally good results in horn fly control
in test cattle through the use of Ronnel mineral blocks. These blocks are still
in the experimental stage.

3. Optional Market Treatment—As few livestock farms are equipped with
good cattle working chutes, stanchions, dipping vats or efficient spray equip-
ment, we recommend for the benefit of livestock growers and feeders that
supervised livestock markets be encouraged to maintain suitable equipment
for the necessary veterinary treatment of animals for internal and external
parasites, such as screwworms, scabies, lice, ticks, flies, stomach worms, etc.
Such action helps prevent the spread of parasites and to increase growers’
profit.

4. Low Level Phenothiazine Feeding—As the continuous low level free
choice pasture feeding of finely ground phenothiazine palatable mineral
mixture to beef cattle is a practical, economical method of aiding in horn fly
and internal parasite control. We recommend that the United States Depart-
ment of Agriculture make the necessary tests to determine most effective uses
of this practical labor saving program.

We suggest that studies be made to determine the effectiveness of pheno-
thiazine and approved systemics like Ronnel fed free choice to pasture cattle
in killing larvae in cattle droppings of both the horn fly and the face fly.

5. Need Control of Horse and Face Flies—Up to the present little practical
help has been given to cattlemen in the control of tabanids or horse flies.
These flies are an important factor in spreading disease and preventing
economical production in beef and dairy cattle. The face fly, found nine years
ago, on cattle in the New England states, is now found in many eastern states
as well as west of the Mississippi River.

We recommend that the United States Department of Agriculture render
livestock growers the much needed assistance by developing safe practical
methods for the control of these destructive annoying flies. Materials that can
be fed free choice, applied by a back rubber, sprayed or drenched or injected
will be welcomed by cattlemen. The livestock industry is looking for a simple,
effective method of application, like the feeding of the material in palatable
block form.
INTRODUCTION

Most of the infectious diseases caused by acid-fast microorganisms tend to be host specific. Indeed, some of the mycobacteria are obligate parasites and cannot be cultivated in vitro and cannot be transmitted to heterologous hosts. Examples of such extreme host-specificity are leprosy, murine leprosy, lepra bulbolorum, mycobacterial infection of wood pigeons and so-called skin tuberculosis in cattle (1). Some acid-fast bacteria which can be grown on artificial media, such as Mycobacterium paratuberculosis, Myco. ulcerans and Myco. microti, have a narrow range of hosts so that experimental studies are limited by the lack of suitable susceptible laboratory animals. Similarly, investigations on certain Mycobacterium spp. from lesions in man are handicapped because many of these microorganisms are not pathogenic for the common laboratory animals (2).

The tubercle bacilli, however, are not restricted to a few hosts. They are able to cause disease in a variety of animals. The terms, “human,” “bovine” and “avian” tubercle bacilli (respectively, Mycobacterium tuberculosis, Myco. bovis and Myco. avium), indicate the primary host of each type, but these designations are by no means indicative of host-specificity. The two mammalian types, human and bovine, can cause disease in a large number of different hosts, including man, farm animals, household pets and wild animals. The avian type is the cause of tuberculosis in domestic and wild fowl, and in addition is able to cause serious tuberculous infection in many kinds of mammals.

It has been said that if tuberculosis were eradicated from man, cattle and fowls, it would tend to disappear from all other species (3). Evidence for this is available. In Denmark the number of instances of bovine tubercle bacillus infection in man has decreased as the disease in cattle has been virtually eradicated (4). Because swine are susceptible to human, bovine and also to avian tubercle bacilli, the relative incidence of infection with these three types of organisms may reflect the incidence of tuberculosis in man, cattle and poultry. Limited data indicate that tuberculosis in swine caused by human or bovine tubercle bacilli is uncommon in those countries in which tuberculosis in man and cattle is relatively well controlled. In the United States, tuberculosis in swine has decreased presumably as a result of the decrease of tuberculosis in poultry, a decrease which results in turn from maintenance of all-pullet flocks (5).

* Mayo Clinic, Rochester, Minnesota.
It is the purpose of this presentation to illustrate by a few examples the relatively wide variety of animals susceptible to infection with the mammalian and the avian tubercle bacilli. It will be emphasized that the control of tuberculosis and final eradication of the disease from one species may be dependent upon elimination of tuberculosis from all species. Finally, and perhaps of greatest importance, it will be emphasized that laboratory studies are vital to the success of the objective of detecting infected animals and the source of their infection.

**Mycobacterium Tuberculosis**

It is repeatedly brought to public notice that tuberculosis in the human population is on the wane in the United States. During the 10-year period from 1948 to 1958 the death rate of tuberculosis declined 76.7 percent. This is no reason for complacency; it is estimated that on January 1, 1960, 30,000,000 Americans had tuberculosis. New instances of active disease will develop from these sources of infection to provide more than 200,000 cases of active disease which we may expect within the next five years (6).

Is human tuberculosis a threat to the animal population? In the campaigns for the control and eradication of bovine tuberculosis, the danger to human health of tuberculosis in animals has been emphasized. We need to recognize, too, that the human tubercle bacillus can cause disease in swine, cattle, dogs and various kinds of exotic animals in zoologic gardens.

In our laboratory we have identified human tubercle bacilli in lesions from an elephant, from a sapajou and from tapirs, as well as from such primates as the orangutan, lemur, and chimpanzee and from many monkeys. Tuberculosis is a very important problem among primates; in the majority of cases the disease has been caused by human tubercle bacilli (7). Cats are relatively resistant to infection with human-type tubercle bacilli, but dogs may be victims of the disease. Accounts have been given of the demonstration of tuberculous infection in dogs living in households with tuberculous persons (8, 9).

Tuberculosis of swine produced by the human type of tubercle bacillus appears to be related to the opportunity among swine for exposure to tuberculous persons. In a number of instances the infection has been traced to the feeding of swine with raw garbage from hospitals (5). Of particular interest to this discussion is the role played by human tuberculosis in sensitizing cattle to tuberculin. Tuberculin reactions in cattle caused by infection with the human strain of tubercle bacillus cannot be considered as either false or nonspecific. The tuberculin used in the United States for the testing of cattle is prepared from cultures of human tubercle bacilli. For practical purposes, it may be said that there is complete cross-sensitivity between human and bovine tubercle bacilli (10).

Tubercle bacilli of the human type have a low capacity to produce progressive tuberculous disease in cattle, but it has been repeatedly shown that sensitivity to tuberculin will develop. In Finland it was found that in many
cases, reactions in the absence of any visible lesions were attributable to infection from tuberculous caretakers (11). In the Island of Jersey a law was passed requiring that farm workers in contact with cattle be examined by roentgenography. It had been found that of 100 cattle in contact with tuberculous persons, 50 were tuberculin reactors (12). A recent report (13) from Great Britain describes a study of five herds of cattle in which bacteriologic examination of the animals and of the caretakers revealed that human-type tubercle bacilli were responsible for tuberculin reactors.

No consideration will be given here to the very important public-health aspect of tuberculosis in animals caused by human-type tubercle bacilli. We are concerned with identification of the type of tubercle bacillus and the sources of infection in the case of animals. This information is needed for the planning and execution of programs for control. Circumstantial evidence that animals have been exposed to tuberculous persons is of interest and has value in epidemiologic investigations. However, only by appropriate laboratory procedures can human-type tubercle bacilli actually be identified.

At present we have no data on the occurrence of infection with human tuberculosis in cattle. We may suspect that in some "problem herds" the disease could be caused by infection from human sources. The cooperation of laboratory and field workers and health authorities is needed. When unsuspected tuberculin reactions occur, medical assistance is needed for the detection of human sources of infection. It should be required that instances of tuberculosis in animals be reported to the appropriate local health agency. Conversely, the diagnosis of active tuberculosis in persons having contact with animals should be reported to the livestock sanitary officials. It is obvious that no tuberculous person should be permitted to work with or have any contact with animals. We need to be concerned with protecting the animal population as well as preventing transmission of tuberculosis from animals to man.

**MYCOBACTERIUM BOVIS**

Bovine tuberculosis is a rare disease in the United States. In 1959 there were only 91 carcasses with tuberculous lesions extensive enough to require condemnation among 17,310,870 cattle (exclusive of tuberculin reactors) slaughtered under federal supervision (14). This fortunate status can be maintained and improved only by continued efforts. Bovine tuberculosis has been practically eliminated as a threat to swine in the United States, whereas it was once considered to be a major infectious disease (5).

In zoologic gardens bovine tuberculosis may be a serious hazard to certain exotic animals. During the past decade in our laboratory bovine tubercle bacilli have been isolated from tuberculous lesions in such herbivores as the giraffe, eland, elk and rhinoceros, and from such animals as the coatimundi, cheetah, sapajou, orangutan and monkeys of many kinds. In one zoologic garden bovine tubercle bacilli were identified as the cause of tuberculosis in 10 animals within a two-year period. The various species infected included a tapir, a guanaco, several elands, a peccary, an okapi and a giraffe.
Where bovine tuberculosis still exists, cats become infected presumably by consuming raw milk. A compilation of reports up to 1945 disclosed that bovine tubercle bacilli were identified in 96 percent of 147 instances of tuberculosis in cats; in the remaining cases the disease was caused by the human type (15). In Switzerland it was found that tuberculosis was diagnosed in 6.3 percent of 1278 cats during a six-year period; 29 of these were examined bacteriologically and in each case the bovine tubercle bacillus was identified (16). In Germany an outbreak of tuberculosis in a herd of cattle was ascribed to two tuberculous cats on the premises infected with bovine tubercle bacilli (16).

Mention has been made of the danger to cattle of persons who have tuberculosis of the human type. Attention should be directed to the possibility that persons with pulmonary tuberculosis of bovine origin are particularly dangerous to cattle. We know nothing about the incidence of bovine tuberculosis in the human population in the United States. It is reasonable to expect that it does exist, particularly among older people. An account has been given of a farmer who contracted pulmonary tuberculosis from his cattle and who subsequently infected three replacement herds within a period of three years (17). In Denmark it is thought that reinfection of cattle with bovine tubercle bacilli may continue as long as there are persons with bovine tuberculosis (4). Cooperation is needed among public health officials, laboratory workers and those responsible for the control of tuberculosis in animals to determine whether in the United States sporadic instances of tuberculosis in cattle result from contact with persons having bovine tuberculosis. Tuberculosis in cattle or other animals cannot be finally controlled unless the disease in man is controlled.

MYCOBACTERIUM AVIUM

It may be said that the avian tubercle bacillus has a wider range of hosts than any other mycobacterium. In addition to being the cause of tuberculous disease in many kinds of avian species, it is capable of infecting a variety of different mammals, including man. In the table are listed some of the species for which the pathogenicity of avian tubercle bacilli is known. Avian tubercle bacilli have been recovered from tuberculous lesions in marsupials, deer, monkeys and goats, and from a large number of exotic birds in zoologic gardens (3, 18).

The economic importance of avian tuberculosis exists not only in the danger to poultry but also in the ease with which the disease is transmitted to swine. As mentioned previously, the avian-type tubercle bacillus is the most common cause of tuberculosis in swine. In the United States in 1957 the incidence in slaughtered swine varied from 3.5 percent in the north central states to 1.3 percent in the southeastern states (5). The lesions of avian tuberculosis in swine usually are limited to lymph nodes of the alimentary canal, but instances of generalized lesions have been seen (18).

In cattle, avian tuberculosis has been found to produce extensive lesions, but in the majority of instances the lesions are limited and tend to regress (19).
The importance of avian tuberculosis in cattle is principally the cross-sensitization with the routine tuberculin test for bovine tuberculosis (20). No extensive bacteriologic studies have been done to determine the cause of tuberculosis or tuberculin reactions in cattle in the United States. An examination of 36 tuberculin reactors in 1939 disclosed that three, or nine percent, had avian tubercle bacilli (21). In Denmark in 1952 (22) it was reported that avian tubercle bacilli were found in 14 percent of specimens from 2,286 tuberculin-positive cattle; 54 percent had bovine and eight specimens had human-type tubercle bacilli. The remainder had tuberculosis lesions of the mesenteric nodes from which no tubercle bacilli were recovered. The specificity of mammalian as compared to avian tuberculin in cattle is only relative. Results of simultaneous tests with avian and mammalian tuberculin are not reliable in herds badly infected with bovine tuberculosis (3).

Avian tubercle bacilli have been recovered from lesions in man only a few times, a fact which suggests that man has a high resistance to this infection. In a review in 1953 of the world literature on avian tuberculosis in man, it was concluded that there were only 39 cases of confirmed disease plus 24 others in which the diagnosis was not certain (23). Four additional cases have been recorded in which avian tubercle bacilli were identified by bacteriologic procedures (24).

At present little information is available concerning the incidence of tuberculosis in poultry, swine and cattle. That the disease is on the decrease is suggested by the gradual lessening of the incidence of tuberculosis in swine, a suggestion which is based only on the data from meat-inspection reports. Without adequate laboratory studies directed toward identifying the causes of granulomatous lesions in animals, the value of meat-inspection data remains in doubt.

**COMMENT AND RECOMMENDATIONS**

The contact between different species of animals on farms, in zoologic gardens and in households provides opportunity for the transmission of tuberculosis from one kind of animal to another. The control and eventual elimination of tuberculosis in one species are dependent on elimination of tuberculosis in all species.

Avian tuberculosis is presumed to be of economic importance to the poultry industry, although the exact incidence of the disease in fowl is not known. This disease also is important because of the wide range of hosts which may be infected with it. The avian tubercle bacillus is the most common cause of tuberculosis in swine in the United States. Within recent years a number of outbreaks of avian tuberculosis in mink have been found. It is presumed from limited studies in the United States that infection with avian tubercle bacilli may be responsible for so-called no-visible-lesion reactors among cattle. Indeed, generalized tuberculosis caused by avian tubercle bacilli has been seen in cattle. It is recommended that efforts be expanded to eradicate tuberculosis from poultry. This will eliminate the
risk of avian tuberculosis among swine and other animals, and perhaps reduce the incidence of so-called nonspecific tuberculin reactions in cattle.

It is natural, in an anthropocentric society, that efforts are directed toward protecting the human population against tuberculosis of animal origin. It should be recognized, however, that the close contact between man and animals provides opportunity for transmission of tuberculous infection from man to various species of animals. We are therefore faced with the need of protecting animals against tuberculous persons. Human type tubercle bacilli have been isolated from tuberculous lesions in swine, in cattle, in pets such as dogs and monkeys, and from lesions in animals displayed in shows and zoologic gardens.

Human type tubercle bacilli may sensitize cattle to the routine tuberculin test. Just how often this sensitization may be responsible for so-called problem herds we have no way of knowing. Only by an organized effort to study this problem will any information be obtained. It is recommended that the local public health authorities be notified in all instances in which tuberculosis is found in animals. Similarly, the disclosure of active tuberculous disease in animal caretakers should be brought to the notice of the livestock sanitary officials concerned.

Finally, it is recommended that bacteriologic examinations be made for the purpose of identifying the type of tubercle bacilli in lesions of animals. A program of control is on a firmer basis if the source of infection can be traced. This is more readily accomplished if the infection is known to be of avian, human or bovine origin.

Bacteriologic studies may disclose causes other than tuberculosis for granulomatous processes in animals. It is still a matter of dispute and conjecture whether or not some agent other than tubercle bacilli may sensitize cattle to tuberculin. Only by adequate and appropriate bacteriologic studies will any information be gained on the vexing problem of the so-called no-visible-lesion reactors.

### TABLE I

**Pathogenicity of Avian Tubercle Bacilli for Various Species***

<table>
<thead>
<tr>
<th>Bird</th>
<th>Susceptibility</th>
<th>Animal</th>
<th>Susceptibility</th>
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<td>Rabbit</td>
<td>Marked</td>
</tr>
<tr>
<td>Pheasant</td>
<td>Marked</td>
<td>Swine</td>
<td>Moderate</td>
</tr>
<tr>
<td>Turkey</td>
<td>Marked</td>
<td>Mink</td>
<td>Moderate</td>
</tr>
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<td>Moderate</td>
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<td>Mouse</td>
<td>Slight</td>
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<tr>
<td>Canary</td>
<td>Moderate</td>
<td>Cat</td>
<td>Not susceptible</td>
</tr>
</tbody>
</table>

* Taken in part from Feldman.18
REFERENCES


At this stage in the tuberculosis eradication program, it seems most important that we should carefully review our position with this disease. Let us examine for a few moments what has been accomplished, where we stand today, and contemplate on where we are going.

I am sure many of you can recall the early years of the eradication program activities and of what must have seemed, at the time, of the monumental task ahead. I understand that when Dr. John A. Kiernan was asked what time would it take to gain the eradication of tuberculosis from livestock that his reply was, "There absolutely are no grounds upon which a reasonable estimate can be made of the number of years it will take to eradicate this disease. All one can do is make a guess as to the time, and it is my belief that if the nation succeeds in eradicating tuberculosis in 50 years, it will be one of the greatest heritages our successors will have handed down to them."

Forty-three years have passed since Doctor Kiernan’s statement was made. Indeed a great deal has been done to bring this disease to a low ebb, however, the goal of the program is eradication, not just holding tuberculosis to a low incidence.

During the past decade, at this present date, and in the immediate years ahead we are in the most difficult and challenging era of the history of the program. Much of the glamour and publicity connected with success have gone and we are faced with the drudgery connected with the final clean-up. In addition to being in a difficult era, we are in a dangerous era—as we have learned during the past five years. In dealing with tuberculosis, we either continue to progress or we regress—and we could regress even more rapidly than any of us might think. This era is indeed one of opportunity—that of gaining to the fullest the heritage that has been handed down—that of getting the job of eradication done.

It appears that the main problem involving the final success of the program is one of attitude. The attitude of determination on the part of everyone to get this job done.

The basic concepts upon which this program was founded are sound. The history of progress since the inception of the program has proven them sound.

In review, these basic concepts are as follows:

1. Periodically tuberculin testing all cattle
2. Removal of reactors to slaughter

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3. Thorough cleaning and disinfection of all infected premises
4. Tracing of movements into and from infected herds to find where the infection originated or where it may have spread.

Might we ask ourselves at this time—are we making the best and fullest use of all the tools and knowledge we presently have at hand in carrying out these concepts? This question seems uppermost as we review the current status of the program today.

During the fiscal year 1960, a total of 9,439,706 cattle were tested for tuberculosis to find 14,149 reactors. The number of cattle tested in fiscal year 1960 represents an increase of 1,252,545 over the number tested during the previous fiscal year. The percentage of reactors found in 1960 was 0.15.

Let us now look at Figure I. Most of you are familiar with this particular slide which Doctor Ranney has used in previous reports to the Association. We have now brought this up to date. You will note that the horizontal bars represent the calculated infection in the United States projected on the basis of a complete test on all of the cattle population. The challenge today is shown by the 152,280 infected animals that remain undetected throughout the country.
As many of you know, the Tuberculosis Staff of the Animal Disease Eradication Division made a survey this past year to determine the number of herds throughout the country in which gross lesions typical of bovine type infection were found in reactors disclosed repeatedly on several retests—truly problem herds and ones which merit special attention. These were called "Red Flag Herds." On July 1, 1960, there were 346 Red Flag Herds reported in 23 states and in numbers ranging from one to over 100 such herds.

Another study of interest to the Association was made by the Tuberculosis Staff on September 1, 1960. This concerned a study on the number of cattle tested for reaccreditation based on the latest data on file for each county in the United States. It was found that 65 percent of all the counties were submitted on the basis of a test of less than 25 percent of the cattle population. There were 13 percent submitted on a test of from 26 to 50 percent of the cattle 5 percent were on a total of from 51 to 75 percent; 3 percent were from 76 to 99 percent; and 14 percent received a 100 percent test.

As we all look forward to new research work to aid us in obtaining improved means and methods in diagnosis—and as we look forward to the results of comprehensive studies in the epidemiology of the many types of tuberculosis to help us in eliminating the many sources of infection, we nevertheless must ask ourselves—Are we using our present tools and knowledge to best advantage? Think of this in terms of these figures; 152,280 undetected infected animals; of 346 Red Flag Herds in 23 states; and of 65 percent of the counties reaccredited on a minimum survey basis.

**APPLYING TESTS EFFECTIVELY**

The key to providing further advances in disease eradication at this time lies in effective testing procedures. Effective testing measures, of course, are viewed from two aspects—one being the individual herd test and the other or broader aspect involving the series of tests made to reaccredit an area.

In a further step toward uniformity of testing techniques on the herd and individual animal basis, the tuberculosis staff has provided this past month, a detailed outline of instructions and procedures for conducting tuberculosis tests. There is actually nothing in this outline which is startlingly new in procedures. These are essentially restatements of basic standards which have been in existence for many years, however, for purposes of clarity, it was deemed advisable to set forth this basic information concerning the conduct of the test.

In 1958 there were 63 counties which were more than a year overdue; in 1959 there were 32 and now there are none. Counties overdue less than a year, in 1958 there were 243, in 1959 there were 145 and in 1960 there are 134. An immediate goal for program progress should be the reduction of those remaining 134 overdue counties as quickly as possible until no county in the country is in this status or becomes in this status without being taken off the accredited list.
STATE-FED. COOPERATIVE TUBERCULOSIS ERADICATION

CASE FINDING AIDS

Those of us who are devoted to the job of eradicating tuberculosis often have looked with some tinge of envy to the vast coverage of herds which the Brucella Ring Test has brought to the Brucellosis Eradication Program. However, we do gain a tremendous benefit in addition to our testing program through the cattle that are slaughtered on straight kill at federal, state, and municipal establishments and in which evidence of tuberculosis is found. Much has been done to perfect tracing operations on these animals. Much remains to be done to further perfect this aid to the program.

During fiscal year 1960, 610 reactor cattle were removed from 99 herds as a result of follow up on tuberculosis lesion cases reported on regular kill. In reviewing the successfully completed cases, the following favorable factors stood out, either singly or in combinations:

1. The lesion case found on straight kill was identified by ear tag number or other means and this was recorded at time of slaughter.
2. The report was promptly submitted to the proper officials.
3. Records of eartag numbers were available.
4. Records at stockyards or local auction sales were adequate.
5. Dealers cooperated in furnishing records and information.
6. Cooperation was evident between the meat inspector, the packer-buyer, stockyards officials, and state and federal officials.
7. There was a prompt energetic investigation.

Unfortunately these favorable factors are not always present, as evidenced by the 69 cases that were not successfully traced out of the total of 400 reported last year.

Illustrative of the problems encountered in effectively following up all cases. Two are reported here—one of which was successfully concluded—the other not resolved. Specimens from both of these cases were sent to the laboratory and a presumptive diagnosis of tuberculosis was made.

One case began with concise animal identification by eartag number. Prompt tracing procedures were carried out and the herd of origin was tested. At this time it was found that the originating animal found on slaughter had been purchased from a neighbor about four years before, therefore a test was also applied to the neighbor's herd. Observations of the test revealed no reactors in the herd of immediate origin, however, five reactors were found in the neighbor's herd. All five reactors were found on slaughter to have advanced tuberculosis. Five days and 424 miles of travel were involved by personnel in successfully following up this case.

The second case concerns a cow—dressed weight of 650 pounds, which was included in a group of 49 cattle purchased from five commission firms operating at a midwestern stockyard. The veterinary meat inspector reported that "no identification marks were available as hides were removed before carcass reached the inspector." The 49 cattle had been shipped by various consignors, including dealers, in two states. Numerous investigations were
carried out. Finally, eight herds, 88 cattle were tested in one state, and 10 herds, 680 cattle were tested in the other state. Personnel spent a total of 33 days and 3,842 miles of travel. There was certainly no lack of effort, but this case was lost—and more important—the infected herd still remains undetected—even though this clue brought us close.

It is apparent that much more needs to be done regarding individual animal identification; more needs to be done concerning maintenance of proper records by anyone involved in the movement of livestock; and more needs to be done regarding cooperation between all persons concerned in these investigations.

OTHER TYPES OF TUBERCULOSIS

In working toward eradication of tuberculosis, we must become increasingly vigilant with this disease in all species of animals including poultry. We must become more aware of these other types of tuberculosis in dealing with a problem herd situation.

A veterinarian applying a herd tuberculin test has three primary responsibilities—one facet is case finding—locating the infected herd and the affected individuals within it. Secondly, he is responsible for containing or isolating the infection through quarantine measures—and thirdly, he is responsible for successful elimination of the infection and its source.

The veterinarian in the field is confronted with the problem; however, that, to enable him to effectively eliminate the source of infection in a problem herd situation, he should be armed with knowledge as to the type of tuberculosis he is dealing with. Ordinarily, without laboratory aid, he does not have this knowledge at hand, in that, the diagnostic responses to tuberculin that he observes may have been induced by mycobacteria, other than bovine tubercle bacilli. This problem was set forth most eloquently recently when Dr. William H. Feldman stated in the American Review of Respiratory Diseases, “One of the most urgent and potentially fruitful problems for research in tuberculosis of man and of animals is that embracing the reciprocal relationship of the tuberculin reaction to hypersensitivity induced by mycobacteria other than true tubercle bacilli.”

As many of you here know, a two-day Conference on Tuberculosis for Laboratory Diagnosticians was held just before this meeting of the Association. This conference should do much toward gaining more activity in tuberculosis laboratory diagnostic work so that we will be in a better position to deal with tuberculosis in the various species of animals.

There is a great deal to be learned regarding the interspecies relationship of the various types of tuberculosis. The work, both in research and in epidemiology now in progress in Michigan, Wisconsin, Utah, and at Ames, Iowa, may be of great benefit to the over-all eradication program in this respect.

There is, however, a great deal that can be done right now regarding tuberculosis in other species. An example of this is with avian tuberculosis.
Whenever, reactors are found in herds of cattle, it is imperative, at such premises, that a check should be made into the possibility of poultry as the source of infection. It is rather surprising to find how little testing of poultry is actually done these days.

Figure II shows those states where testing of poultry took place this past fiscal year. This is based on monthly reports received by the Tuberculosis Staff. You will note that only 16 states reported tests made on poultry. It is of interest to note that all of these 16 states reporting testing also reported the disclosure of infected flocks. The total flocks tested was only 109; of these, 45 (or 41 percent) were found to contain infected birds.

PARATUBERCULOSIS

Those of us who are working in tuberculosis eradication are of course necessarily interested in paratuberculosis. There were 20 states in which testing was reported. A total of 485 herds were tested and 89 of these were found to contain reactor animals. As we look to the future, this disease is one that should receive greater attention.

THE GOAL IN HUMAN TUBERCULOSIS

I am sure that this Association will be interested in knowing that those concerned with human tuberculosis have established a new goal. The Arden
House Conference, a meeting of 19 national experts at Harriman, New York, in December, 1959, was called by the United States Public Health Service and the National Tuberculosis Association to take stock of human tuberculosis and to plan for the future.

There were 12 recommendations made at that conference and a goal was set—the elimination of tuberculosis. In accomplishing this goal, the work ahead in human medicine lies in eliminating the reservoir of infection and the tuberculin test will provide one of the major tools in finding this reservoir.

It is most interesting to compare these recommendations with our current program and to find that basically there is only one major difference—that being treatment.

All veterinarians and livestock sanitary officials interested in tuberculosis eradication work should join and take part in local tuberculosis associations. A great deal of benefit can be derived from a mutual exchange of information and experiences.

THE YEARS AHEAD

As we look forward to the coming years, the skeptics may ask, “Can it really be done?”, in realizing the many obstacles that must be overcome. On the other hand, the optimist replies, “Anything can be done if the American public sets its mind to it.”

What do we mean when we speak about this goal of eradication? Perhaps this was best set forth when Dr. Fred L. Soper stated in the August, 1960, issue of the Journal of American Veterinary Medical Association: “Eradication . . . refers to the complete disappearance of all sources of infection of a given disease agent, so that no recurrence of that disease is possible, even in the absence of all preventative measures.”

To all livestock growers, this is the beginning of the end of a long fight. If you and I believe it can be done, then it will be done.

The goal is set—eradication. The time is now—the ’60’s.

The challenge is ours.
REPORT OF COMMITTEE ON TUBERCULOSIS


Your Committee on Tuberculosis has been offered several worthwhile suggestions for changes and improvements for the Uniform Methods and Rules. Each has been given due consideration. We are pleased that the two conferences on tuberculosis for laboratory diagnosticians were so successful. Meetings of national scope specifically on tuberculosis not only tend to congeal the thinking on this important disease but stimulates an interest needed to overcome the complacency that has existed for so many years. There is every reason to believe that additional laboratory facilities will become available to assist in more specific diagnoses.

The tuberculosis problems in Michigan and Wisconsin specifically were reviewed by your Committee. The Livestock Sanitary Officials and the livestock industry in these two states are to be commended for their bold, prompt and courageous solution to their common problem. While adhering strictly to the uniform methods and rules every effort is being made utilizing epidemiological statistical and research studies to add information to the knowledge of tuberculosis.

We are concerned, however, that in other parts of the country some feel a need to constantly criticize and/or defend the tuberculin test and even the tuberculin, itself, in public. The intradermal tuberculin test has probably been used more frequently than any other similar test and has done a remarkable job of reducing the incidence of the disease.

The tendency for each veterinarian to feel that he has a right to make an interpretation according to his own standards must cease. Unusual or atypical reactions must be considered potentially dangerous and the animals handled accordingly. Until and unless new techniques and materials are developed the execution and interpretation of the test must be made in strict accordance with the recommendations of the Uniform Methods and Rules. A manual of instructions for the application of the tuberculin test has been incorporated.

There is need for renewed interest in research. The studies in Wisconsin and Michigan are just a start. The goal of eradication of tuberculosis must be uppermost in the minds of all people, although from time to time some seem to want to settle for control on an endless basis. The eradication procedures, however, must be less costly on the long term basis than control and must be realistic enough to recognize the differences in the basic problems under the various conditions in the United States. It is recommended that in
the future a mathematical formula based on cattle population density, cattle movement, and disease incidence be used as a method to find statistically the soundest approach toward locating the specific infection probability for the area to be tested.

This would be an objective statistical approach toward determining the specific number of cattle to be screened in a given area to most efficiently locate infection, and would result in a wiser expenditure of monies and manpower.

The definition of a tuberculosis free area has been thoroughly discussed and it is the unanimous opinion of your Committee that at present such an area could not be practically described without making concessions to the goal of eradication.

In that the minimal age of animals to be tested needs clarification the following definition should be added:

9. “Age of Cattle to be tested”—in infected and accredited herds, all cattle shall be tested. On routine area testing, all cattle six (6) months of age and over shall be tested.

INSTRUCTIONS AND PROCEDURES FOR CONDUCTING TUBERCULIN TESTS

A. Caudal fold and/or vulva

1. Restraint—Cattle being tested must be properly immobilized for a period of time sufficient to permit a careful injection. Stanchioned dairy cattle will normally be restrained by use of a nose-lead or halter. Nose-leads will be washed in a disinfectant solution between animals. Non-stanchioned cattle should be confined by means of a chute, squeeze gate or equally affective means. The injection site (caudal fold and vulva, if used) is to be cleaned with dry cotton. Where necessary, preliminary cleaning of the area may be accomplished by the use of paper or synthetic fiber toweling.

Needles used in applying the tuberculin test will be limited exclusively to those of 26 gauge and 3/8 inch exposure. The syringe, needle and chuck will be maintained in a clean condition by swabbing with cotton and alcohol as necessary. The needle and chuck will be swabbed with alcohol and wiped with dry cotton prior to each injection. (A pledget of cotton saturated with alcohol may be affixed to the left wrist or forearm with a rubber band and renewed as needed. After wiping needle and chuck, drying is accomplished by wiping with the pledget of dry cotton to be used in preparing the injection site.

In making the injection, care should be taken to assure intradermal inoculation of tuberculin. Substantially all of the exposed needle should be inserted, withdrawn slightly, and 0.1 cc. of tuberculin injected. (A dosage of 0.2 cc. will be used in those herds in which advanced tuberculosis is known to exist.)
2. *Observation and Interpretation of the Test*—Careful consideration must be given to the observation of each animal. It is essential that the site of injection be carefully examined by palpation, 72 hours following injection. Visual observation, alone, of unconfined animals can in no way be considered an acceptable procedure. Reactions to tuberculin may be either hard and circumscribed or soft and infiltrated with no distinct line of demarcation and may be of various sizes, from those hardly perceptible to those as large as a human fist or larger.

3. *Reporting symbols*—Each deviation from normal shall be recorded in the observation column of the test report, using the following symbols:
   a. PP—A “PP” (Pin-point) shall be recorded for any circumscribed swelling which is less than a P₁ reaction.
   b. P₁—To be recorded for circumscribed swellings the size of a small pea (diameter $\frac{3}{16}$ of an inch). Larger circumscribed swellings shall be reported as “P₂”, “P₃”, “P₄”, etc., the figures referring to 2, 3 or 4 times the diameter of a small pea.
   c. X—“X” shall be recorded for any diffuse thickening or swelling which is less than the thickness of the normal fold of skin.
   d. X₂—To be recorded for a diffuse swelling in which the disturbance is equal to twice the thickness of the normal fold of skin. Larger diffuse swellings should be recorded as “X₃”, “X₄”, “X₅”, etc., the figures referring to 3, 4 or 5 times the thickness of the normal fold of skin.

4. *Classification*
   a. *Reactors*—“R”
      Animals showing P₁–X₂ or greater response to tuberculin on routine test should be classed as reactors unless in the professional judgment of the testing veterinarian a suspect classification is justified. For all such cases, reasons for the alternate classification must be clearly noted on the test chart.
      Only in rare instances should such animals be classified as other than reactors. For herds with a history of lesions in the head glands and/or thoracic area, animals showing any tissue disturbance at the site of injection should be classified as reactors.
   b. *Suspects*—“S”
      This classification is to be used for animals showing any response at point of injection not classified as a reactor, with the exception noted below.
   c. *Negative*—“N”
      Animals showing no tissue disturbance at site of injection will be classed as negative. Animals showing a minimal tissue response (pp or x) in herds which contain no reactors on the current test, and in which ad-
advanced tuberculosis (gross lesions of tuberculosis) has not been demonstrated on previous tests may be considered as deviators and classed as negative. This does not apply to retests of accredited herds, herds in the process of accreditation or tests on animals intended for sale, show or interstate shipment. (Decisions will be based upon the professional judgment of the testing veterinarian.)

5. **Retests**—All retests will be applied by full-time regulatory veterinarians insofar as available personnel will permit.

B. Cervical Test (Restricted use):

1. Where on any one test, 20 percent of the animals tested react (at least two reactors) and where 50 percent of such reactors reveal lesions in the head glands and/or thoracic area, the cervical test should be applied.

2. The cervical test will be applied in herds where advanced tuberculosis persists in spite of repeated caudal tests.

3. The cervical test may be applied when directed by the local state and federal officials if reactors revealing lesions are disclosed following a test of the herd.

4. The cervical test is to be used only as outlined above by experienced full-time salaried veterinarians as follows:

   a. 0.2 cc. of mammalian tuberculin to be injected into the cervical area of each animal regardless of age. Caudal injection may also be used if 60 days have elapsed since prior test.

   b. Readings are to be made and recorded at the 48th and 72nd hours following injection. (A 24-hour reading should be added in herds where tuberculosis persists.) All animals with deviations from normal are to be classed as reactors. Retests are to be made on previously unused cervical skin areas. (At least 4 inches from a previously used site) at two week, or longer, intervals not to exceed 60 days until two successive negative tests have been obtained. On these retests all animals showing three mm. or more ($\frac{2}{10}$ inch) increase in skin thickness should be classed as reactors.

   c. Following two negative cervical tests, the interval between the tests to be lengthened to 60-90 days and regular caudal fold testing procedures carried out using 0.1 cc. of tuberculin.
FIELD CONTROL OF AVIAN ENCEPHALOMYELITIS

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For nearly twenty years after avian encephalomyelitis (AE), sometimes referred to as epidemic tremor, was first recognized by Jones (7) in 1932, the epidemiology of the disease remained a mystery. Although reports of laboratory investigations with the causative virus (13, 14, 21) appeared from time to time, the literature on AE was scant, and only in the past five years has interest in it resulted in the publication of epidemiological work of much significance (17, 19, 20).

During the dormant phase of investigational studies the disease was reported to occur in many widely separated areas of the world. These reports were summarized in 1942 by Jungherr and Minard (10) and again in 1952 by Feibel, et al. (6). The latter work marked the beginning of renewed interest on the part of researchers in AE.

Any attempt to calculate the cost to the poultry industry of the losses due to AE during this period must be based on conjecture, but it is reasonable to assume that they would total in the hundreds of thousands of dollars. The additional cost to breeders and hatcherymen, if they followed the recommendations of the poultry textbooks of that era to "dispose of infected flocks and start over with clean stock" would have added an equally staggering sum to the total bill. A major defect of this recommendation was that it offered no clue to a method by which "clean" stock could be located and recognized. Although put forth with the best of intentions by veterinarians and poultry pathologists, the advice to dispose of affected breeding flocks was based on partial information and has been shown by later studies (17, 20) to be the complete opposite of the most appropriate procedure for controlling outbreaks of AE in chicks.

Early Investigations

In fact, some of the very earliest investigations with AE provided evidence that survivors of this disease are unlikely to become carriers capable of transmitting the virus to their progeny. In a study reported by Jones (8) (1934) and by Bottorff (1) (1936), an effort was made to hatch additional AE-infected chicks from parents which had previously produced infected chicks. Among the progeny from five hatches no AE was diagnosed. Cockerels and pullets which survived the original outbreak were also mated but their chicks from five hatches likewise showed no evidence of AE. Later, in 1941, Van Roekel et al. (21) reported that no positive evidence of AE was detected among 696 chicks hatched over a three-month period from eggs.
laid by 26 ataxic pullets. These pullets had survived an attack of AE and came from several different flocks. A second hatching trial conducted several months later with part of the birds used in the first trial also failed to produce infected chicks.

That artificial exposure to the AE virus might actually provide protection against subsequent challenge by intracerebral inoculation with the virus was apparently first recognized by Jungherr and Minard (10) in 1942. They reported that of eight non-cerebral routes tried, the chicks inoculated in the peritoneal cavity were not susceptible to intracerebral re-inoculation. It would appear, therefore, that from the earliest studies of this disease we have evidence to suggest that it can be controlled by a program of judicious exposure of prospective breeding stock to the live virus.

Embryo Studies

Attempts by Kligler and Olitsky (12) in 1940 to cultivate the AE virus in chicken embryos failed, although these authors reported success in obtaining multiplication of the virus in tissue culture. Van Roekel, et al. (21) in 1941, described investigations in which it was demonstrated that the AE virus is capable of multiplication in embryos. In nine different trials a total of 807 fresh unincubated eggs were inoculated with the virus, and in 12 additional trials a total of 370 embryos 10 to 12 days old were also inoculated, and allowed to hatch. A high percentage of the hatched chicks in both groups of tests manifested evidence of the disease at hatching time or developed signs of the infection by the time they were eight days of age.

In 1956 Wills and Moulthrop (22) described a practical technique for propagating the AE virus in the embryo, and Jungherr et al. (11) reported the histopathology produced by an embryo-propagated virus (18). The work described by these investigators opened the way for fruitful inquiries by others into the epidemiology of the disease. Sumner, et al. (19) in 1957, demonstrated a technique for differentiating between resistant and susceptible embryos, thus providing a means for identifying breeder flocks which have been exposed to the AE virus. These authors conducted susceptibility titrations on fertile eggs collected from 119 flocks situated in widely separated areas of the United States. Only four of these flocks produced embryos which allowed the development of maximum titers. These and other findings led Jungherr (9) in 1958 to state: “The combined observations changed the entire former concept of the disease. We now know that (a) we are dealing with a widespread infection of laying flocks which goes unnoticed in many instances; (b) epidemic tremor (AE) in chicks occurs in the progeny of the exceptional, susceptible flock; and (c) we are dealing with a disease impossible to eradicate and only attackable through proper vaccination.”

These conclusions on the wide distribution of the virus were confirmed by Taylor and Schelling (20) who in 1960 reported the development of a simplified test for immunity of breeder flocks to AE, and the results of its application to more than 2,000 flocks throughout the United States and Canada. The test is based on the failure of embryos to support the growth of AE virus
injected into them. These authors found an increasing percentage of resistant flocks, ranging from 56.8 percent at five months of age to 95.7 percent at 13 to 18 months.

First Successful Field Control of AE

The first report of successful field control of AE was published in 1955 by Schaaf and Lamoreux (17). They described an extensive outbreak of the disease which occurred in the winter of 1949-1950 among chicks from a large breeder-hatchery organization in California in which they observed that affected chicks were hatched mainly from eggs laid by pullet breeders, and that chicks from hens a year or more older were for the most part free of signs of the disease. This was true even when pullets and old hens were mingled in the same pedigree mating pens. They concluded from this that the older hens had in some manner been exposed previously to the disease and were thereby made incapable of transmitting it to their offspring. This conclusion was strengthened when after a short period (one to three weeks) the pullet flocks ceased producing affected chicks.

Working from this premise the breeder-hatchery ignored the recommendation to dispose of the affected breeders and, the following year, purposely exposed the next generation of prospective breeding stock to the live virus of AE. This was done late in the growing period before sexual maturity, using a five percent brain suspension of the virus which had been isolated from chicks affected in the outbreak of the previous winter and propagated in day-old chicks by intracerebral inoculation. The exposure virus was administered by wing web puncture.

These authors reported that since the inception of the controlled exposure program there had been no serious recurrence of the disease among baby chicks under conditions where:

1. the survivors of the 1949-1950 outbreak of AE were retained as breeders;
2. the live virus of AE had been systematically distributed to young chickens before they reached maturity and to older ones at the time of their annual molt;
3. the disease was known to be present in active form as shown by the demonstration of clinical signs by a few of the inoculated chickens and by the re-isolation of AE virus from them, and
4. eggs were hatched every week of each year so that no important recurrence of AE could have passed unnoticed.

In two different experiments, young chickens inoculated in the wing web with 25 CLD\textsubscript{50} quantities of AE virus later suffered less than half the mortality observed among controls following intracerebral challenge with 250 CLD\textsubscript{50} quantities of the virus.

Throughout the years since 1950 this procedure of vaccinating prospective breeding flocks with the live virus of AE in order to prevent egg transmission
of the disease to their subsequent progeny has been followed. Up to the present time (September, 1960) nearly two million doses of the vaccine have been administered with no major outbreak of AE in more than 55 million pullet chicks hatched from the vaccinated breeders.

This vaccination program has served as very valuable insurance at low cost. The results have been good under the system of management used. The mortality attributable to vaccination has been low, much less than one percent, and protection has been provided against natural outbreaks at seasons of the year which might make them tremendously expensive, both to the breeder and to the poultrymen who anticipated receiving chicks. Such customers, who have depended on receiving chicks as scheduled, would often find it difficult on short notice to replace their orders elsewhere for chicks of similar quality.

**Immunization with BPL-inactivated Virus**

When vaccination of immature breeding stock for AE introduces the risk of exposing susceptible breeding hens to live virus, an effective inactivated virus is needed. Schaaf (15) in 1959, described the preparation and use in immature chickens of five experimental AE vaccines inactivated with beta-propiolactone (BPL). The best protection was obtained with a vaccine having a pH of 7.4 and containing 6.25 percent aluminum hydroxide and 12.5 percent brain tissue. This vaccine did not lose its effectiveness after storage at 4°C. for fourteen months. Each of the vaccines which contained the aluminum hydroxide adjuvant effectively induced immunity, as shown by protection, complete in one instance, from intracerebral challenge with live AE virus five to seven weeks after vaccination.

Using the serum-neutralization test (3) to determine the levels of immunity obtained, Calnek and Taylor (5) in 1960 concluded that consistent and high responses to a BPL-inactivated AE virus can be obtained providing dosage is adequate. A schedule of two doses at five-week intervals of 200,000 to 400,000 EID_{50} produced neutralization indices of 2.0 to 3.0.

**Oral Exposure to AE Virus**

In a further report on immunization for the control of AE Schaaf (16) in 1958 described a vaccination experiment in which two groups of chickens 46 days of age received varying doses of AE virus administered per os. When challenged intracerebrally eight weeks following vaccination, 81 percent of the isolated controls succumbed, while only 45 percent of the contact controls and 26 percent and 13 percent respectively of the two vaccinated groups did so. Although none of the orally exposed chickens developed signs of the disease as a result of such exposure, the fact that more than one half of the uninoculated chickens in contact with them resisted challenge is evidence that sufficient contact spread of the virus occurred to induce useful immunity, and suggests that oral exposure to the virus may be a safe and convenient method for the immunization of susceptible breeding flocks.

A similar trial reported in 1959 by Calnek and Jehnich (4) produced comparable results. These authors noted that AE may be essentially an
infection of the intestinal tract and that ingestion may be the primary means of spread. They suggested that administration of the AE virus by the oral route or the inoculation of a small percentage of the birds within a group would make this means of immunization extremely feasible for floor raised birds.

That the AE virus may normally occur in nature as an enteric virus was suggested by a report of Jungherr and Minard (10) who in 1942 described the results of intracerebral inoculation of test chickens with filtrates of feces obtained from hens of breeding flocks with, and without, a history of producing chicks affected with AE. No symptoms of AE were observed as a result of the inoculation, but histopathologic lesions in the central nervous system were produced which were indistinguishable from those associated with the AE virus. The percentage of inoculated chickens with induced lesions was fairly high in both groups, but was much higher in the group inoculated with fecal filtrates from flocks with a known history of AE infection. An additional report of interest here is that by Burke et al. (2) which describes the isolation of a cytopathogenic agent from a clinically negative flock. This agent was neutralized by AE-immune serum, and conversely, was capable of producing an antiserum which neutralized AE virus. These authors found their agent to be still viable after 22 hours at 56°C. Resistance to prolonged heating is also a characteristic of the AE virus. In a test conducted several years ago, the writer found a saline suspension of AE virus to retain its infectivity for chicks after being held at 37°C for two weeks.

Discussion

In retrospect, it seems rather remarkable that so many years went by from the discovery and isolation of the AE virus, until control measures were developed. A brief discussion here of some of the factors involved in this delay may be worthy of consideration in dealing with other diseases which represent current problems to the poultry industry.

One of the most baffling characteristics of AE has been its erratic occurrence, which led to conflicting evidence concerning the possibility of repeated outbreaks of AE in the same hens. A complete history of the outbreak tracing the chicks back to the flock unit which produced the eggs from which they were hatched is extremely important, and would serve to clarify this point. Hatcheries should be urged to hatch separately the eggs received from different supply flocks and to keep complete records of the distribution of chicks obtained from them.

The natural tendency of AE to express itself in a biennial cycle had the effect of reducing the urgency of the problem it presented to the individual hatcheryman, and perhaps also to the research worker. But where the research worker seeking academic knowledge often can avoid a decision, the poultry breeder must decide from information available at the moment what is best for him to do, and then act on his decision. It is not likely that many poultry breeders confronted with an outbreak of AE among chicks from
valuable and often irreplaceable lines of breeding would follow the recommendation to “dispose of infected breeding stock,” because to do so would put them out of the breeding business. Instead, they would weather the outbreak, cull heavily, and rear the survivors. The exposure to the disease as chicks caused these survivors to become immune, and the hatcheryman would not be troubled by outbreaks among chicks hatched from them the following season. These second generation chicks, if reared to maturity without exposure to the AE virus, would produce AE-infected chicks if they were exposed to the virus after their eggs were being saved for hatching, and the biennial cycle of infection would continue. This natural cycle is not always readily apparent, of course, as changes in management methods and introduction of new stock exert varying influences on the visible expression of the infection.

The remedy for this confusion, and for the increasing economic loss from this disease, lies in vaccination, or controlled exposure, of prospective breeders with the live virus of AE at a time in the life of the chicken when such exposure is economically most feasible.

In the light of our current knowledge of the epidemiology of AE it seems unlikely that anyone would now raise a serious objection to its control by industry-wide vaccination. Whether such control is brought about by individual wingweb vaccination with chick-brain propagated virus, which has been used successfully by some poultry breeders for as long as ten years, or by mass exposure to embryo-propagated “enteric” strains of the virus, is of little real significance. The decision is rather one between the philosophy of indefinite postponement and that of useful action.

It is recognized that strains of AE virus exist which represent extremes of pathogenicity. Some field strains of AE virus in current use by private breeders as wingweb vaccines are perhaps more “neurotropic” than might be considered optimum, while other strains (23) are so mild as to be rather difficult to propagate by intracerebral injection into chicks from immune hens. To what extent these and the embryo-propagated “enteric” strains used as oral vaccines will protect breeding chickens against all the unknown but suspected field strains in existence, remains to be explored. It is not suggested, however, that incomplete knowledge on this and other points should delay the use of currently available immunization procedures, which are known to be safe and effective.

Conclusion

The research summarized here has clearly shown that the AE virus is widely spread, probably universal in its distribution among poultry flocks. It is known to cause expensive, inconvenient, and often embarrassing outbreaks of disease in chicks. A live virus vaccine, administered to parent breeders at an appropriate time, has been shown to prevent the disease in their chicks. In deciding whether or not to approve the use of such vaccines in their present stage of development we, as individual veterinarians, must be careful to distinguish between justified risk and unjustified conservatism.
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THE CONTROL OF AVIAN ENCEPHALOMYELITIS BY THE APPLICATION OF SUSCEPTIBILITY TEST AND VACCINATION BY THE WATER PROCEDURE


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Epidemic tremors (avian encephalomyelitis) has been recognized as a problem in the poultry industry since 1930. It has been an allusive problem, often striking without warning the chicks produced by a given hatchery but frequently disappearing from the scene not to appear in subsequent hatchings of the same hatchery for months or years.

With the development during the past decade or so of large national poultry-breeding organizations, a great deal of attention has been given to this disease through the facilities and personnel provided by these establishments. Coupled with continued attention to this disease by a number of workers at universities, much has been learned about this condition and we find ourselves approaching satisfactory means of control.

A survey of the diagnostic reports of state laboratories would give one the impression that epidemic tremors is a frequent problem, but that its distribution around the country is rather limited. This misconception stems from a number of factors. First of all, a large percentage of the problems encountered in chicks is never submitted to a laboratory as a diagnosis is readily made in the field. Secondly, because it is an asymptomatic infection when contracted by growing or adult birds the vast majority of infections go unnoticed. Only when a chick contracts the infection by egg-transmission or by contact in the first few days of life are the typical symptoms of tremors and ataxia seen. Therefore, in areas where only seasonal hatching is being done, and because of a high infection rate, most hatchery supply flocks have contracted the infection prior to the time that eggs are being saved for hatching. This means that they are immunized before hatching eggs are used, and no chicks are produced with the disease.

A survey conducted on a large number of flocks across the United States and Canada demonstrated that this disease is, indeed, widespread to the extent that over 95 percent of all flocks 18 months of age or older have gone through the asymptomatic infection. This survey was performed by means of a susceptibility test using eggs laid by the flocks being tested. Following exposure to this disease not only are there circulating antibodies in the bloodstream but a very high level of antibodies are incorporated in the yolk of all eggs laid. These antibodies are present in sufficient quantities to neutralize an egg-adapted epidemic tremor virus when inoculated at five days of incubation. Eggs from a susceptible flock which has had no experience with the disease will support the growth of the virus and by 14 days of incubation definite changes in the embryo will be observed. The details of this test have
been reported elsewhere (1). Not only were the results of this test important to us in demonstrating the widespread nature of this disease, but it provided for us our first line of defense in controlling this condition. Wherever possible, only those supply flocks which had demonstrated resistance to the disease (previous exposure) were used for hatching purposes. In not a single case were chicks with epidemic tremors produced from a flock which had been demonstrated by this test as having been resistant. At the same time a high incidence of the disease was experienced in hatchings from susceptible flocks and flocks of unknown status. The reliability of this test rivals even the more conventional test for immunity such as the serum-neutralization test in the case of this disease.

In most cases in a given associate hatchery, it has been a simple matter to do all the hatching from resistant flocks and refrain from the use of susceptible flocks until such time by re-testing these, too, have picked up the asymptomatic infection and are safe to use. However, in some cases a given associate hatchery may have only a small number of supply flocks, all (or nearly all) of which by chance have not contracted the disease and are susceptible. This has occasionally created local shortages of hatching eggs, and it became apparent that a reliable vaccination procedure for these susceptible flocks would be helpful.

A number of procedures have been studied for immunization against epidemic tremors. A procedure which has attracted our attention has been the oral administration of the virus by the drinking water method. This procedure has some definite advantages over individual bird inoculation, especially where adult birds are concerned, because of the labor involved and the stress of handling imposed by the latter procedure. We have restricted our cultural procedures to the embryonated-egg rather than the living chick brain as the chances of contamination by extraneous virus are not nearly as great.

Several strains of virus have been employed in these studies in an attempt to select a strain which is mild in its effect upon the bird but which quickly produces immunity. These tests are still in progress and appear most promising. Through the cooperation of state and federal authorities a large number of flocks are being vaccinated this season by the drinking water procedure. All of these flocks have been shown to be susceptible by the above-mentioned test and are followed by retesting to determine if immunity is developed. The effect on egg production, spread to adjacent birds, and egg transmission of the vaccine virus are also being followed.

We believe that in the not-too-distant future a safe and reliable vaccine will be available for use by the industry.

REFERENCE

ORNITHOSIS IN TURKEYS

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Ornithosis, or psittacosis if we accept these terms as being synonymous, has gained a great deal of attention during the last decade. This attention has been stimulated by an increase of the disease in man from psittacine birds and outbreaks of ornithosis in poultry processing personnel. The disease was not well known until what is now referred to as the pandemic of 1929-30, which was a widespread occurrence of ornithosis in man in 12 different countries attributed to the exportation of infected parrots from South America. Within a few years, it was shown that this reservoir had changed and that psittacine relatives of the parrots mainly parakeets, were disseminators of psittacosis and latent infection in aviaries over the world was found to be an established fact.

These findings appeared to constitute the entire problem of psittacosis until 1938, when Haagen and Maurer (1) reported that the fulmar or petrel was the source of human infection in the Faroe Islands. This finding altered the concept that psittacine birds were the sole source of ornithosis. In 1940, Coles (2) in South Africa isolated ornithosis virus from pigeons. Subsequently, it was established that ornithosis was of world wide distribution in pigeons. The virus was then isolated from ducks in New York and California and from chickens in Michigan and California. In 1951, Irons, et al. (3) reported on ornithosis among workers of a poultry dressing plant in Texas. The virus was subsequently isolated by Boney, et al. (4) from turkeys in 1952. The Texas outbreak was followed by others in that state and in other states. These findings have established a wide host range of the psittacosis lymphogranuloma viruses and while a great increase in disease contracted from nonpsittacine sources has occurred, an increase in psittacine originated infections also had occurred from the association between man and infected caged pet birds. This increased infection was in part attributed to the tremendous growth of the pet bird industry which has been estimated to have increased from three to 15 million during the years 1940 to 1956, and in part to the relaxation of restriction of interstate movement of psittacine birds in 1951.

The host range of ornithosis has steadily increased in number and kind of avian hosts. Meyer (5) states that at least 98 species of birds have been proven to be naturally infected with ornithosis. In addition to the wide range of infection in avian species, isolations of viruses morphologically and antigenically indistinguishable from those in avian hosts have been made in the following mammalian species, mice, cats, calves, sheep, and opossum.

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The relationship of these viruses to avian strains is still not completely determined.

The true incidence of ornithosis in man is difficult to obtain because the disease syndrome is not uniquely specific. Ornithosis is one of several pulmonary diseases which from clinical manifestations, might be diagnosed and reported as several entities, viral pneumonia, atypically pneumonia, influenza, or colds. Frequently, unless there is reason to suspect otherwise, no investigation is made into the matter except for treatment of the patient. While psittacine birds and turkeys currently are considered the main source of infection, we still have a large number of cases of ornithosis in man for which no source of infection is available, and contact with birds or poultry is unknown.

Frequently the disease in poultry is described by analogy to infection in psittacine cage pets. Such an approach has serious limitations. Ornithosis in poultry constitutes an important reservoir and deserves to be considered as a distinct subject in itself. The position of ornithosis as a poultry disease has not been completely defined. One reason for this, perhaps, is that the disease is considered of infrequent occurrence and to cause severe disease in those birds when it occurs. While it is true that acute ornithosis is at times severe, in pigeons, ducks, and turkeys, there is evidence that this is not always the case and perhaps that this is the exception rather than the rule. Enzootic ornithosis among chickens, ducks, turkeys, pheasants, and pigeons has been shown to occur as inapparent disease producing no visible symptoms and few or no pathological lesions.

Enzootic ornithosis in pigeons has been documented as being world wide in distribution and widely disseminated in the United States. The high incidence of subclinical and mild to severe infection among pigeon farmers and dealers attest to its existence. Adult pigeons can be latently infected and shed the virus. They transmit the virus in the nests to young. Some of these infected nestlings may develop into a chronic symptomless carrier state unless the host parasite balance is upset. These birds may remain visibly healthy while shedding large quantities of virus into their environment.

Ornithosis is enzootic in ducks in the United States and has, for the most part, been clinically inapparent. Severe epizootics of ornithosis have been observed in Czechoslovakia resulting in high mortality. While the ornithosis virus has been recovered from ducks of all ages, the disease appears to be of most importance to young ducklings where the mortality and pathological lesions are most severe. The coexistence of other diseases such as virus hepatitis, pasteurellosis, salmonellosis, infectious serositis, often complicates its diagnosis. In chickens, little is known about ornithosis except that it has occurred in subclinical cases.

Ornithosis was first discovered in turkeys as a result of a poultry processing plant outbreak in 1948 in Texas. The virus was isolated from the lungs and kidneys of a turkey with no gross lesions. Since that original outbreak, serious epizootics have occurred in Texas, New Jersey, California, Oregon,
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and Vancouver. From these outbreaks have been isolated viruses which we call the highly toxic strains of ornithosis or the highly virulent strains.

While we have not heard a great deal about these virulent outbreaks until recently, little has been mentioned of the occurrence of milder strains of ornithosis due to viruses of low virulence. Ornithosis agents have been recovered from turkeys in California that were characterized by a relatively low pathogenicity for turkeys and laboratory animals (6, 7). In these instances, the disease in the flock was mild, and losses were few (less than one percent). There were no known infections among persons handling infected birds on the premises or during processing. In Wisconsin, an outbreak of ornithosis occurred in personnel in a turkey processing plant from two turkey flocks (8). The isolates were of the low virulent type. Most of the human cases were mild or inapparent in nature. The ornithosis in these turkeys presented no unusual morbidity or mortality although occasionally transitory respiratory symptoms were noted. Flocks in this area under observation for three years failed to show any unusual appearance suggestive of ornithosis.

A serological survey of employees of a chicken and turkey processing plant in an area where no evidence of ornithosis epidemics (past or present) have occurred revealed a high percentage of complement fixing antibodies to ornithosis (9). Such evidence lends support to the presence of agents of low virulence in areas where they have not been reported.

In contrast to these mild outbreaks, the virulent strains stand out, such as the Oregon outbreak which resulted in 86 cases with two deaths (10). This outbreak furnished information on the clinical course of a virulent epizootic of turkey ornithosis. The exact date of onset is unknown, however, in early December minor losses in the flock were attributed to fowl cholera or erysipelas. Laboratory tests did not confirm this. Of approximately 1,800 turkeys processed from this group, 79 were condemned because of air sac lesions. Losses in the remaining birds increased in January and by March, losses ranged from 20 percent to 30 percent. Total losses were 2,000 birds.

The turkeys exhibited clinical symptoms of anorexia, fever, depression and other general symptoms. Necropsy of sacrificed birds presented the following lesions: Inflammation of serosal surfaces, air sac infection, slightly enlarged livers covered with fibrinous film. However, few spleens were noted enlarged. The pericardium of almost all birds showed involvement. Myocarditis was evident, pneumonia and pulmonary edema were present. At processing, five weeks after the acute epizootic was arrested by treatment, birds showed chronic heart and liver lesions resulting in condemnation of a large percent of these organs.

Necropsy findings of experimentally infected turkeys with a virus of low virulence (at the Ohio Agricultural Experiment Station) by comparison resembled those of the virulent strains but are not quite as extensive (12, 13). The lesions observed were primarily pericarditis, perihepatitis, and aero-sacculites (see Figures 1, 2, and 3). Lesions are not consistently found in birds infected with viruses of low virulence and the same has been noted in
FIGURE 1. Air sac from turkey killed nine days after inoculation with ornithosis virus. Notice the air sac is greatly thickened, cloudy, and edematous.

FIGURE 2. Liver from turkey killed nine days after inoculation with ornithosis virus. Notice the film of cellular exudate covering its surface.
FIELD outbreaks with virulent strains (11). Additional pathology found may vary such as epicardial petechiae, edema and pneumonia. The birds do not manifest clinical signs of infection except if infected with heavy doses at 10 days of age or earlier. The similarity of lesions between chronic respiratory disease, pasteurellosis and erysipelas emphasize the importance of a differential diagnosis. Gross pathological or clinical observations are not sufficiently distinctive to be pathognomonic.

Most ornithosis like viruses can be isolated readily by inoculations of the suspect material by intraperitoneal or intranasal inoculation of mice or by inoculation of embryonated eggs. However, three serial blind passages of mouse or egg material are necessary to exclude the presence of the virus from suspect material. The lesions and mortality seen in mice will depend upon the toxicity of the virus in question. Highly virulent strains will result in the death of mice within a short period while strains of low toxicity may result only in a chronic illness with slight or no mortality (14). They generally cause a fibrinous exudate in the peritoneal cavity with an enlargement of the spleen and liver (see Figures 4 and 5).
FIGURE 4. Ascites in mouse inoculated with low virulence virus of ornithosis nine days previously. Control on right.

FIGURE 5. Photograph of spleen and liver of a mouse killed nine days after inoculation with ornithosis virus of low virulence.
ORNITHOSIS IN TURKEYS

The differences of virulence of agents isolated from turkeys from different sources are striking. Very little has been done to learn how these differences affect the ecology of ornithosis. Ornithosis of low or moderate virulence should appear to be best adapted to survive in nature or its natural host. There is good evidence that this situation exists in pigeons; perhaps a similar situation is present in turkeys. The problem of virulence and especially changes in virulence under natural conditions is of great importance.

The pathogenicity among the ornithosis agents isolated from turkeys at different sources has been shown to vary greatly. We have the highly virulent strains which cause variable mortality and the strains of low virulence which cause low mortality or none. Even the less virulent strains have been shown to differ from each other markedly in their effects on laboratory animals. Two California strains of ornithosis agents differed greatly in their infectivity and lethality for pigeons and turkeys (16). One of these strains was 30 times more infective for turkeys, while the other strain was over 525 times more lethal for pigeons. No significant differences were noted in their pathogenicity for chicken embryos, mice, guinea pigs or parakeets.

The viruses of the psittacosis-lymphogranuloma group have been shown to produce toxins when injected via the yolk sac into embryonated eggs. These yolk sacs heavily infected with these viruses and toxins produce variable mortality in mice on intravenous inoculation. Certain of these toxins are highly virulent at high dilutions (1:1,000) and kill 50 percent of the mice within 16 hours. Each virus strain produces its specific toxin and antiserum which will protect against these losses. The toxins have been found to be closely associated with the elementary bodies of the virus and have been used to distinguish between various strains. The highly virulent strains have been found to be the most potent toxin producers. The toxin producing ability of avian isolates has been found to vary considerably.

It is interesting to speculate how poultry became hosts to ornithosis. It seems unlikely that psittacine cage birds imported into the country were the source of the infection. The benign quality of the infection in some poultry suggests a balance of some duration. Until more is known about this disease in poultry, one can only speculate. Information about ornithosis in wild or migratory birds is lacking and is a necessary part of our knowledge if we are to fully understand the epidemiology of ornithosis. The means by which the virus survives between outbreaks is not known. Transmission via the egg has not been demonstrated (14). In view of modern poultry practices of incubator hatched eggs, the adult to young transmission cycle is broken, except for indirect contact on farms where adult birds are kept. Experiments showing that ornithosis can be transmitted by natural means from pigeons to turkeys and the recovery of ornithosis viruses from sparrows nesting near ornithosis-infected turkeys points out possible vectors of this condition (7, 15). Further study is necessary regarding these agents and the mode of transmission before definite conclusions can be drawn.

Control measures for elimination of ornithosis are currently not available, except to apply the basic principles of control for infectious diseases. Once
infection has been diagnosed, treatment of these flocks with antibiotics in the feed and water does reduce mortality and morbidity, but is still uncertain in effectiveness in eliminating the virus (17). Quarantine, treatment with antibiotics, observation and sampling of such flocks to determine when and if they are safe for processing under inspection appears to be a necessary expedient, since the antibiotics have been found to be capable of destroying the virus (18, 19).

Until more information is obtained regarding the epidemiology of this condition, we can only concentrate our attention on the prevention of spread when the disease occurs.

REFERENCES


THE USE OF KILLED VIRUS VACCINE TO CONTROL
NEWCASTLE DISEASE

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The purpose of this report is to emphasize the need for reevaluating the
Newcastle disease (ND) control program in the United States.

Newcastle disease was first officially recognized in the United States in
late 1944 in California. It was reported on the East Coast in 1945 and had
spread to many poultry producing states by 1947. At that time, methods
were urgently needed to reduce the huge economic losses that were caused by
heavy mortality, decreased egg production, lowered feed conversion, increased
numbers of culls, and lowered quality.

Research workers in the United States Department of Agriculture have
always selected one of the following procedures for controlling infectious
diseases of livestock and poultry: 1) prevent disease introduction; 2)
eradicate the disease, especially where it has been recently introduced or
where affected areas are small; 3) use killed immunizing agents associated
with sound management and sanitary practices; and 4) use live immunizing
agents only as a temporary expedient to protect the industry until safer
products can be developed.

Intensive research on ND by the United States Department of Agriculture,
the various state institutions, and private biologic companies began imme-
diately as disease losses appeared. Killed and modified live virus ND
vaccines became available as a result of this research. The killed virus
vaccines produced at that time did not induce immunity of long duration.
However, the live virus vaccines were considerably more efficient. It was left
to the discretion of the livestock sanitary official of each state as to the
advisability of the use of these products. At the present time, there are a few
states that restrict or they do not permit modified live virus ND vaccine. The
killed vaccines are used to a very limited degree. When it became the practice
of simultaneous vaccination against ND and infectious bronchitis with the
live viruses, it was necessary for the producer to obtain a permit for its use
from the state livestock sanitary official.

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D.C.
Since the widespread incidence of ND in the United States, research workers of the United States Department of Agriculture have worked toward developing a killed virus ND vaccine, which would stimulate an immunity of long duration. Such a vaccine has been developed (2) and experimental evidence as well as field trials (3) indicate that it is effective in preventing losses in broilers and egg production in layer flocks.

It is most important that we recognize the disadvantages from using live virus vaccine. The disadvantages have been pointed out many times by competent researchers, among whom are Adler (1), Jungherr and Lubinbuhl (4), Laurie-Rhodes (5), and Stubbs (6). The fact that living vaccines may produce the disease if they are improperly used, that they perpetuate the disease and in many instances are stress agents which precipitate latent infections, particularly air-sac infection, are reasons why their use should not be continued beyond the point of actual necessity. In addition, the use of live ND vaccines in the United States has had a detrimental influence on exports of poultry and poultry products to foreign countries.

Effective control of ND has not been accomplished by using modified live virus vaccines. Their use in the original emergency was fully justified since a satisfactory killed vaccine was not available. Experience has demonstrated that it is more difficult and time consuming to work out the problems associated with effective killed vaccines as compared with modified live virus vaccines.

Within recent years there has been renewed interest among research groups and regulatory officials in the possibility of eradicating ND in the United States. The Northcentral Technical Committee on Avian Diseases initiated a movement aimed at a ND eradication program somewhere within that area. Such a program would necessitate using killed ND virus vaccine. Prohibition or restricted use of other live poultry vaccines may also be desirable or even necessary.

Associated with the initiation of the Federal Poultry Inspection Act on July 1, 1959, there was an increase in poultry condemnations because of respiratory infections. The criteria for these rejections were the same throughout the United States. Much of the losses encountered at this time did not occur previously because of the lack of inspection. Research in recent years (7) has shown that air-sac infection is precipitated by live virus vaccination of poultry.

The State livestock sanitary officials should consider controlling ND through the use of a safe killed vaccine which will not perpetuate the disease nor stimulate latent infection. They should urge commercial biological companies to make available to the poultry industry sufficient quantities of the more efficient killed ND vaccine.
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Integration has markedly influenced most poultry operations throughout this country. Poultry operations such as broiler production and egg laying production have continually become larger operations with a larger number of poultry confined to smaller areas. The major pit-fall to the over-all assembly line integrated program is that of disease and disease control. The industry can control nutrition and management practices, but the disease problem presents the unpredictable which can have a devastating effect upon the over-all economy.

Basic economics are most important in today's poultry operations. Production costs, such as cost per pound of bird, or cost per dozen eggs are the prime prerequisites for survival of poultrymen. Needless to state, diseases and disease control can and do become an important part of production costs, and can be the balance of success or failure for each operation. The very nature of the transition of the poultry industry has increased the disease hazard on poultry operations.

There is an ever-increasing need for more sound disease control programs as well as a need for more expedient accurate diagnosis of disease problems that are presented.

The poultry industry looks to the veterinary profession for help with their problems. Many large segments of the industry have their own veterinarians on their staff, but many more depend upon state, federal, and educational institutions for their aid in programing and diagnosis.

Your Committee would encourage that all such agencies take a more active interest in the needs of the poultry industry, in supplying adequate diagnostic as well as consultative services.

Your Committee commends the editors of Avian Diseases which is published by the American Association of Avian Pathologists on the publication and recommends the use of this source of information to bring about a better understanding of poultry disease problems.

At the present time there is considerable discussion towards eradication of pullorum and paratyphoid disease of poultry. Legislation is encouraged to support such programs, but with the sudden recognition of the presence of salmonella organisms in feed samples the program may meet with a new stumbling block. The awareness of salmonella organisms in feed (which is included in a later section of this report) could well introduce a new problem.
of public health significance. Your Committee recommends that this problem of contaminated feed be thoroughly scrutinized without delay by United States Department of Agriculture before a major situation develops.

In an effort to bring about a uniformity of diagnosis as well as an alertness for poultry diseases, your Committee strongly endorses the establishment of a standardized national morbidity and mortality reporting system with an efficient distribution which would be available to all interested agencies, organizations, and individuals.

The Committee wishes to alert the regulatory and public health officials on the report of ornithosis. This disease has been conclusively demonstrated in a flock of chickens in Oregon. Unfortunately, this disease was first diagnosed in the owner of the flock. This is of considerable concern to the veterinary profession because an agent of such public health significance is making its appearance in another species of poultry, and therefore requires considerable more attention. Regulatory officials should constantly be on the alert and not be led into a false sense of security because ornithosis in turkeys has not been diagnosed with regularity since the Texas, California, and Oregon epornitics which occurred a few years ago.

Your Committee recommends the adoption of the proposed interstate movement regulation of poultry by Agricultural Research Service but strongly recommends that serious consideration be given to the submitting of health certificates to the chief livestock sanitary official of the state of destination.

The condemnation rate in chickens has been emphasized in the preceding year's report. The importance of this problem is re-emphasized. Your Committee recognizes that there is a problem in the form of high condemnation in poultry inspection in broilers, and it has recently been pointed out that a similar situation is developing in turkeys. Your Committee is of the opinion that both problems should receive consideration by the United States Livestock Sanitary Association. The Poultry Committee would be willing to help to whatever extent this situation may need.

Although mycoplasmos (PPLO) has been recognized as an etiological disease entity for considerable numbers of years and a rapid test for its detection has been developed, there is a dire need for availability of a universal standardized antigen of consistent uniformity and performance for diagnosis.

Therefore this Committee strongly recommends that serious and immediate consideration be given to the availability of an efficient antigen because early diagnosis of infection cannot be divorced from the high condemnation rates in the poultry processing plants.

This year's Committee continued its efforts of compiling the latest information concerning poultry diseases and have included this information in our report.
Diseases involving the respiratory tract of chickens are still one of the major problems confronting the poultry industry and contribute to the condemnation of carcasses upon inspection. Investigations of this serious situation revealed that the conditions are caused by a plurality of agents of varying pathogenic potential. Studies indicate that combination of agents irrespective of their disease-producing properties alone results in an infection considerably more severe than is usually produced by the single disease agent. Cross (1) reported that he was able to reproduce a pericarditis and perihepatitis, which is frequently referred to as an “air sac infection,” in chickens when exposed to a combination of infectious bronchitis, mycoplasma, and \textit{E. coli}. Characteristic lesions were reproduceable when a proper sequence of agents were employed, whereas none of the agents were capable of producing the “air sac syndrome” alone.

At least three new agents have been studied, which in all instances are only slightly pathogenic. The infrequency of their isolation during routine diagnosis may be attributed to their low pathogenicity and difficulty or impracticability of isolation with our present methods. Subramanyam and Pomeroy (2), as well as Hansen \textit{et al.} (3), studied the Fahey-Crawley virus to show that it is a very minute, stable agent which causes only the mildest of respiratory signs and has extremely poor immunogenicity. Bankowski \textit{et al.} (4), isolated a similar avirulent agent from the respiratory tract of chickens suffering from the unusually severe outbreak of laryngotracheitis. Burke, Luginbuhl, and Jungherr have demonstrated the presence of a large number of viral agents which are harbored in the intestinal tract of chickens (5, 6) of 103 isolates which were made from predominantly growing birds, at least one agent was believed to be serologically related to avian encephalomyelitis virus. The revelation and importance of the more recent isolates does not necessarily reveal them as the underlying cause for the present respiratory disease complex but strongly points to the necessity of determining the role they may play when found in the presence of other more virulent viruses or bacteria. It is not within the scope of this report to speculate their origin and role in the respiratory disease complex of today.

Newcastle disease (\textit{ND}). A number of reports upon methods that may be employed for characterizing and identifying strains of \textit{ND} virus in relation to their virulence have been reported (7, 8, 9). Kohn and Ebert (10), Asdel and Hanson (11), studied the route traveled by \textit{ND} virus following injection into susceptible chickens, which re-emphasizes the dexterity, invasiveness, and presence of the agent in practically all tissues in the chicken.

Although the immunity to Newcastle disease vaccination has been considerably studied, it is apparent that the immunity to \textit{ND} virus following vaccination is more complex than previously believed (12, 13). Bankowski, \textit{et al.}, showed that the quality of an immunity varies considerably with the product and that protection of the respiratory system, the central nervous system, and the reproductive tract may vary independently of each other and
with the product. Protection of all three systems and the duration of protection to each or all of these systems must be considered in evaluation of a vaccine. In studies with a tissue-culture-propagated vaccine, two doses given at four to five weeks of age and repeated prior to laying resulted in an immunity which protected the three systems in a high percentage of the birds for at least 101 weeks (13). Rosenwald, Hanson, and Brandly (14), studied methods for the detection of agents of high virulence but in low numbers in living vaccines that were produced with the less virulent strains. They concluded that as little as one part in ten million can be detected provided that is passed through embryonating eggs and the infected fluids of the latter inoculated into chickens. They also suggested a numerical expression for characterizing the virulence of a Newcastle disease agent for its characterization study.

*Infectious bronchitis.* The widespread presence of IB has been attested by the recent observation by Raggi and Lee (15), that IB exists in Hawaii. Cunningham (16), in his recent studies on IB virus, stressed the complexity of the agent and the difficulties which may be encountered in diagnosis because of plurality and immunogenic differences among strains. The introduction of tissue culture methods to IB, reported during the past two years, should materially enlighten and simplify the study of this agent in this important disease.

**CHRONIC RESPIRATORY DISEASE AND INFECTIOUS SINUSITIS**

It was found by Olson *et al.* (17), that chlortetracycline at levels of 125, 250 or 375 grams per ton of feed, with and without 0.5 percent terephthalic acid was effective in preventing the retardation in weight associated with chronic respiratory disease. When chlortetracycline was fed at levels of 375 grams per ton of feed along with 0.5 percent terephthalic acid, the development of chronic respiratory disease was prevented. The use of terephthalic acid apparently potentiated the chlortetracycline from two to three times. In another study, Olesink and Van Rockel (18), found that chlortetracycline or oxytetracycline were higher than non-treated-inoculated birds in all but one group. The chlortetracycline and oxytetracycline only slightly inhibited the signs and lesions of the disease.

In a report of disease factors causing condemnation and downgrading of poultry, Wenger (25), noted that chronic respiratory disease and/or respiratory lesions are the largest single cause contributing to condemnations.

In three groups of turkeys infected with mycoplasma, Abbot *et al.* (26), noted a high incidence of air sac lesions in embryos and cull poults, abnormal patterns of seasonal egg production and hatchability, a drop in egg production as well as, various other findings.

Hanson *et al.* (3), Subramanyam and Pomeroy (2), have suggested that the respiratory symptoms in the turkey poults noted may have been due to the Fahey-Crawley virus activating a latent PPLO infection.
Observations were made by Adler et al. (27), on methods of inducing and evaluating immunity against mycoplasma infection in chickens and turkeys. They noted that recovery from the disease confers measurable immunity and that an attenuated culture administered intramuscularly immunized chickens for about two months.

Dodermuth and Gross (19), grew four strains of Mycoplasma gallisepticum (W.S-6, 293 and VB) utilizing a semi-solid agar technique. All strains grew well in this type of medium and produced well isolated colonies which were suitable for comparative morphological studies. In another study, Adler and Berg (20), made a comparative study on the ability of 5 strains of pathogenic mycoplasma to grow in various media. They suggested that for best results, at least two media be used for mycoplasma isolation—one enriched with chicken serum and the other with horse serum.

Kleckner (28), presented evidence which indicates that avian pleuropneumonia-like organisms (PPLO) may be grouped into various serotypes. He also found that strains capable of producing lesions and serologic titers in turkey poult's fell into just one serotype and that none of the non-pathogenic PPLO reacted with this serotype.

Barnes et al. (21), treated young turkeys, experimentally infected with infectious sinusitis, with various dosages of three forms of erythromycin. Turkeys infected with four strains of PPLO responded to erythromycin therapy of 50 mg per sinus.

Smibert et al. (25), inoculated 6-week old turkeys with a pathogenic strain of avian PPLO (MD-2). They found a marked increase in the microbial population in tracheal tissues examined between the seventh and 30th days and an increase in the bacterial population of the lung and air sac tissues from the 10th through the 30th day.

The bacterial flora of the respiratory system of 10 fowl with natural cases of aerosaccitis was studied by Smibert et al. (25), who found that Gram-negative organisms (mostly E. coli) and PPLO were isolated most frequently from the respiratory system. Gram negative organisms (mostly E. coli) were isolated from the pericardial tissue exhibiting lesions.

PASTEURELLOSIS

A study was made by Heddleston and Reisinger to determine the lowest concentration of a suspension of formalin-killed Pasteurella multocida which would produce immunity in chickens. Of the four different concentrations used, no significant differences were noted in the ability to establish a high degree of immunity in chickens, for at least one year. Eighteen out of 19 (95 percent) vaccinated turkeys survived a challenge of virulent Pasteurella multocida while one of 11 (nine percent) of the unvaccinated controls survived a challenge at 21 weeks.

HEMORRHAGIC DISEASE

Dempsey and Sanford (29), studied the effect of various antibiotics on the hemorrhagic condition in chickens. They noted that levels of 10, 100 and
200 gram per ton levels of some antibiotic supplements caused significant increases in hemorrhages; the interaction between crude antibiotics and the Kansas State University corn-soybean oil meal basal diet revealed significant increases in hemorrhages as compared with the same antibiotics in purified diets; crystalline forms of antibiotics caused significantly less hemorrhages than crude feeding grade antibiotics; and crude feeding grade sources of oxytetracycline and zinc bacitracin caused an increase in hemorrhagic severity in comparison with procaine penicillin.

ERYSIPelas

Investigations concerning *Erysipelothrix rhusiopathiae* infection in poultry have virtually come to a standstill in this country. The disease is endemic in many of the concentrated turkey-producing areas in the United States. With proper application of the commercial erysipelas bacterins that are available, the disease in turkeys has been greatly reduced in those areas where it has been a most serious problem, such as in the Pacific Northwest. Outbreaks of this disease, however, in non-vaccinated as well as vaccinated turkeys, testify to the need for further research on this disease. Special attention should be given to epizoolgy with particular emphasis on the reservoirs or “healthy carriers.” The facility with which this organism may change its characteristics is an area that needs immediate and urgent study for a better understanding of the disease in birds. Although the present bacterins are helpful in handling the erysipelas problem, there should be further research to try to develop an immunizing agent that would provide a more stable and longer lasting immunity.

In case of an outbreak, the use of penicillin parenterally as well as orally has been shown to be the most satisfactory treatment for sick birds to check immediate mortality. It, of course, is advisable to inoculate all birds in the flock with erysipelas bacterin at the time the sick birds are treated with penicillin. It is recommended that the bacterin be injected subcutaneously in the dorsal area of the neck one or two inches below the skull. Intramuscular inoculation will establish immunity but the prolonged local reaction may result in condemnation if inoculated birds should perchance be sent to slaughter within eight weeks following inoculation.

The continued reports of *E. rhusiopathiae* infections in chicken flocks should emphasize to diagnosticians the importance of searching for this organism as a cause for disease in this species of animal.

COCCIDIOSIS

Coccidiosis is recognized as one of the most common causes for loss in commercial poultry production. It is known that much loss is the result of lowered egg production or decreased efficiency in developing body weight in meat producing birds. This economic loss is not always as evident to the poultry producer as the dramatic loss due to sudden mortality. That there are many factors that influence the effect of coccidiosis on birds is well
illustrated in the work reported by Erasmus, et al. (31). They demonstrated that, “Although the severity of the coccidiosis was similar in chicks receiving low levels of Vitamin A as compared to chicks receiving higher levels of the vitamin, recovery of surviving chicks, as measured by improved appetites and growth rates, was enhanced as the level of Vitamin A in the diet was increased.”

There are many drugs and combinations of drugs that are helpful in controlling avian coccidiosis. Gardiner (32), reports, “Excellent control of experimentally induced cecal coccidiosis” with a combination of sulfaquinoxaline and aureomycin. On the other hand, he reports, “Terramycin at 166 grams per ton, in conjunction with sulfaquinoxaline at either 0.125 or 0.025 percent gave very unsatisfactory results.” These data emphasize the need for prudence in selecting drugs and combinations for control of avian coccidiosis.

Avian coccidiosis may ultimately yield to artificial immunization as reported by Dickinson, et al. (33), and Bankowski, et al. (34), for the poultry producer that continues his operation on litter. For many years there has been a growing tendency, in some of the dense commercial production areas, to raise poultry on self-cleaning floors of wire or rods. When properly utilized this type of management alone breaks the coccidia cycle to an extent that clinical coccidiosis is no longer a problem.

PARASITES

Frazier (30), reported on three field trials which were performed to test the effectiveness of Hygromycin (Lilly) as an anthelmintic in laying hens. One trial indicated that Hygromycin was effective against both C. obsignata (Columbiae) and A. galli when fed at the rate of eight grams per ton of feed for eight weeks. Another trial indicated a partial control of C. obsignata and complete control of H. gallinae.

Weisbroth (35), made a study of the comparative morphology of the northern fowl mite and the red mite of poultry to determine a means of rapid differentiation between the species. The anal plate structure of the northern fowl mite was found tear drop in outline while that of the red mite was keystone in shape. Comparative photographs were included in the article to demonstrate this point of differentiation.

A study of the effect of various additional stresses on the severity of histomoniasis was made by Welter (36), who found that starvation of turkeys suffering from histomoniasis increased the incidence of liver lesions, although this was not found to be the case with chickens. Other stress factors which did not show a significant effect upon histomoniasis in chickens and turkeys were anaemia, exposure to temperature extremes, coccidia infection, fowl pox vaccination, cortisone injection and splenectomy.

A previously unreported roundworm infection (Superfamily-Trichuroidea, genus-Capillaria) in the epidermis of the bill of ducks was reported by Ferri et al. (37).
Foster et al. (38), made a study on the effect of continuous feeding of hygromycin as a poultry anthelmintic and its effect on laying house performance. They noted that the continuous feeding of hygromycin in a practical type diet resulted in a higher rate of production and improvement of feed efficiency and that for optimum results, the hygromycin B should be included in both the growing and the laying ration.

SALMONELLOSIS

Pullorum Disease

Progress continues in the reduction of the incidence of Pullorum disease and fowl typhoid. A committee of the American Association of Avian Pathologists conducted a study of the Pullorum-Typhoid Control programs in the 47 states. The results of the study were as follows:

1. The Pullorum Control Program is administered in 20 states by an independent board, in 18 states by a Division of the Department of Agriculture, in five states by Livestock Sanitary Board and in four states by University department. The Pullorum Control Program is administered by veterinary personnel in only a few states.

2. Twenty of the agencies administering the Pullorum Control Programs have broad regulatory power in the area of poultry diseases. In the other 27, the Official State Agency has regulatory power restricted to pullorum disease.

3. Eleven states have a compulsory Pullorum Control Program involving chicken and turkey breeding flocks.

4. Twenty-two states have some type of an importation regulation concerning poultry diseases.

5. Forty states reported outbreaks of pullorum disease in chickens or turkeys in 1959.


7. Only 10 states quarantine flocks that have been found infected with pullorum disease and fowl typhoid.

8. Twenty-one states require the reporting of outbreaks of pullorum disease and fowl typhoid by private diagnostic laboratories and practicing veterinarians.

9. Twenty-five states have health regulations governing exhibitions of poultry, but none of them require the birds to originate from U. S. Pullorum-Typhoid Clean flocks or its equivalent.

There is interest in the development of a program to eradicate pullorum disease. Each state should strengthen its program and consider the following areas:

1. Support the adoption of an inter-state regulation on pullorum disease and fowl typhoid.
(2) Obtain 100 percent participation of all chicken and turkey breeding flocks under the pullorum control program.

(3) Require the reporting of outbreaks of pullorum disease and fowl typhoid by diagnostic laboratories and veterinarians.

(4) Infected flocks be quarantined and the marketing of such flocks be in a plant under state or federal supervision.

(5) Poultry consigned to public exhibitions originate from U. S. Pullorum-Typhoid Clean flocks or its equivalent.

Van Roekel (39), has extensively reviewed the present Pullorum Disease Status and has made specific recommendations to be considered in the development of an eradication program.

Jacobs et al. (40), reported on the hatchery spread of pullorum disease through debeaking and recommended a procedure to sterilize the equipment.

Bruner (41) reported on the typing of S. pullorum isolates and found that many isolates were mixtures of intermediate and standard forms. The variant form appeared to be more stable than standard form.

Lancaster et al. (42), studied dust and fluff samples from commercial hatcheries in British Columbia and found considerable variation between hatcheries and samples from the same hatchery.

At the National Plans Conference in Athens, Georgia, in June, 1960, hatchery and farm sanitation programs were proposed and accepted as guides for participants of the plans. More emphasis was placed on the use of formaldehyde fumigation at higher levels. The results of the Pullorum-Typhoid Control Program are indicated in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Chickens Tested Number</th>
<th>Reactors</th>
<th>Percent</th>
<th>Turkeys Tested Number</th>
<th>Reactors</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>40,614,440</td>
<td>18,369</td>
<td>0.07</td>
<td>3,838,755</td>
<td>2,170</td>
<td>0.06</td>
</tr>
<tr>
<td>1958</td>
<td>36,756,760</td>
<td>11,423</td>
<td>0.03</td>
<td>3,509,580</td>
<td>640</td>
<td>0.018</td>
</tr>
<tr>
<td>1959</td>
<td>40,716,266</td>
<td>9,920</td>
<td>0.024</td>
<td>3,940,033</td>
<td>437</td>
<td>0.01</td>
</tr>
<tr>
<td>1960</td>
<td>37,030,568</td>
<td>6,812</td>
<td>0.018</td>
<td>3,510,154</td>
<td>243</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Paratyphoid**

The flocks were designated as typhi-murium tested and no reactors found on the basis of the serological test. Ninety-seven percent of the 623 flocks in the program qualified on this basis. Other states (Wisconsin and Iowa) have a similar voluntary program available to the industry.

Henderson et al. (43), studied the pathogenicity of seven different Salmonella serotypes in chicks and the mortality varied from two percent to 80 percent. The presence of Salmonellas in animal and poultry feedstuffs was
emphasized in the 1959 report and additional information indicates that feed contamination presents a serious problem in attaining the goal of eradication of the pullorum disease, fowl typhoid and paratyphoid infections.

**SALMONELLA—ISOLATION FROM FEED SAMPLES**

Many reports have been made regarding the isolation of Salmonella species from feed (44, 45, 46, 47, 48, 49). In one study, Erwin (46) examined 206 test samples of commercially prepared poultry feed and found 27 samples to produce Salmonella-like colonies on differential media. Boyer, et al. (47), isolated 9 serotypes of Salmonella from turkey feed and meat scraps. In a survey involving 666 feed samples from 22 states and found 166 samples to show the presence of Salmonella. These positive samples came from 16 states and included 41 Salmonella serotypes. Your Committee feels that further exploration of this problem is needed in protecting the industry from such sources of infection.

**ORNITHOSIS**

Ornithosis was diagnosed in one flock of 100 chickens during 1960. This case occurred in Oregon and was first brought to light when Ornithosis was diagnosed in a man who had been under a physician’s treatment for over three years for chronic respiratory embarrassment and heart insufficiency. The physician drew a blood sample in November, 1959 from which the diagnosis of Ornithosis was made. At the same time that the man suffered from his respiratory condition, his chicken flock was also afflicted with a disease of respiratory nature.

Ornithosis was diagnosed by laboratory means in the chicken flock and it has been disposed of by slaughter. Ornithosis was tentatively diagnosed in a turkey processing plant employee, according to a report submitted by a practicing physician in Oregon. The suspected source of this infection was an Oregon turkey flock of 950 birds slaughtered on August 9, 1960. The casualty, a turkey picker, was working in the processing plant on the date this flock was slaughtered. The turkey flock suffered a condemnation loss of 62 birds classified under septicemia.

By late 1959, 17 cases (52) of psittacosis in humans, by contact with infected turkeys, 10 of them serologically confirmed during a four-month period in 1956, were found in Wisconsin and Minnesota. Apparently healthy turkeys harbored the virus which was transmitted during processing. This indicates a possibility that there is a large reservoir of low virulence ornithosis virus in the states of Wisconsin and Minnesota.

Five unrelated cases (51) of psittacosis of humans were reported in California. Four of the cases were people exposed to pet parakeets or pigeons. The fifth case involved a laboratory worker who had been working with psittacosis virus.

Recent work on the treatment of ornithosis and psittacosis gives some encouragement in this field. One report (52) states that psittacosis in
parakeets can be controlled by tetracycline compounds when fed in impregnated hulled millet seeds or by injections. Recoveries have been almost complete but not always immediate.

Moore and Watkins (53) compared the effectiveness of chlortetracycline and oxytetracycline at different levels in eight-week-old turkeys inoculated with the Jo strain of ornithosis. Oxytetracycline was unsatisfactory at 200 Gm/ton of feed concentration, whereas chlortetracycline eliminated the ornithosis agent from the tissues used at this level. Oxytetracycline prevented isolation of the organism when fed at the rate of 600 Gm/ton in the feed for three weeks. Low calcium diet was the most effective antibiotic “enhancer,” but it had no effect on the efficacy of treatment under conditions of this study. After three weeks of treatment, neither of the drugs was enhanced.

Daugherty, et al. (54), investigated titrations of a single lot of frozen psittacosis virus in eggs which were obtained from different flocks and from the same flock at different times. Measurement of survival time after injection of virus into different groups of eggs revealed that the eggs may vary significantly both in uniformity of response and in mean survival time after injection of a given dose of virus. This makes it impossible to compare psittacosis virus titrations based on survival time alone when titrations are done at different periods. A method of titration is described which allows estimates of potency based on survival time of eggs following injection of a single dose of virus, combined with a standard LD 50 titration.

**AVIAN INFECTIOUS HEPATITIS**

Research work continues on Avian Infectious Hepatitis, both for the viral and vibrionic diseases. Sevian et al. (55), report that the continuous administration of furazolidone at a 100 gm. level (per ton) in the feed affords satisfactory protection against the vibrio, but, they recommend this only on farms where the disease is a constant problem. They further state that in most instances, birds with hepatitis respond well to therapeutic doses.

**PROPOSED FEDERAL REGULATION ON THE INTERSTATE MOVEMENT OF POULTRY**

In 1958 the National Turkey and Poultry Improvement Plans Conference adopted a resolution requesting that the United States Department of Agriculture, Agricultural Research Service Animal Disease Eradication Division, develop minimum federal regulations for interstate movement of poultry.

Animal Disease Eradication Division submitted the proposed regulation to the June, 1960, meeting of the National Poultry and Turkey Improvement Plans Conference. The proposed restrictions were generally endorsed with recommendations that minor changes be made.

Since that time, a copy of the proposed regulation has been mailed to a nation-wide mailing list of people and organizations allied to the poultry industry. All individuals wishing to submit comments have been invited to do so by Animal Disease Eradication Division.
At the time of this writing, the Director of the Animal Disease Eradication Division is considering all comments regarding the proposed regulation and after due deliberation will either endorse the proposed regulation, make certain changes and republish it, or if he does not concur in the suggested alternatives, the matter will be dropped.

HYSTEROESIS

Hysteriosis is a new condition of poultry which has been recently brought to the attention of the poultry pathologists. Whether or not this is a disease caused by endocrine imbalance, nutrition, management or genetics it is to be solved. It is expressed primarily in white Leghorn replacement chickens and also young laying hens. The birds tend to mill around and attempt to hide as if frightened without reason for such behavior. It was noted most often in large groups of birds on litter, though it has occurred on wire in some instances. Whether or not this is a “new” disease or simply a problem of transitory importance remains to be seen.

REFERENCES


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A REVIEW OF THE Q FEVER PROBLEM WITH PARTICULAR REFERENCE TO THE PRESENT STATUS OF THE INFECTION IN GEORGIA

L. E. STARR, D.V.M., Ph.D.* and W. C. HENNING, B.S.†

INTRODUCTION

Q fever, a rickettsial disease, which usually occurs as a silent infection in ruminants, cattle, sheep, and goats, was first reported in 1937 in Australia. Subsequent investigations have revealed infection in many other species of wildlife, primarily in ticks and probably other insects and birds. Man is susceptible to infection under certain conditions, usually from contact with soil, dust, contaminated hides, wool, et cetera, but apparently not to infection by ticks. The disease is now known to be world-wide in distribution.

A survey reported in this paper indicates widespread infection in dairy cattle in Georgia but no clinical or serological evidence of human infection. This does not preclude cases in man in the future. In view of the high incidence and wide distribution of Q fever in cattle in Georgia, human susceptibility and the intimate occupational relationship of dairymen and veterinarians with cattle and abattoir employees with animal products, physicians and health departments should be alert to the possibility of human cases in Georgia.

ETIOLOGY

Q fever was first reported by Derrick (1) in 1937 in connection with an outbreak of an acute febrile illness among abattoir employees in Brisbane, Australia. Burnet and Freeman succeeded in isolating the etiological agent, later called Rickettsia burneti, in mice from guinea pig liver sent them by Derrick. Because the disease was not well understood, therefore posing a “query,” it was named Q fever.

A filter-passing but otherwise rickettsia-like agent was isolated from the dog tick Dermacentor andersoni in Montana by Davis and Cox in 1938 (2). Investigation of a human laboratory infection indicated that this Montana tick rickettsia was identical with R. burneti. Subsequently the name of the Q fever rickettsia was changed officially to Coxiella burneti because of its filter-passing property and its remarkable resistance to desiccation and heat.

Parker et al. (3) define the Q fever organism as a pleomorphic rickettsial organism which possesses the ability to pass filters that are impermeable to ordinary bacteria and to rickettsiae in general. It stains sharply and deeply with Giemsa’s and Machiavella’s method but not well with ordinary dyes used to demonstrate bacteria. It is observed as small lanceolate rods, bipolar

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rods, diplobacilli and occasionally as segmented filamentous forms. Measurements are approximately 0.25 μ in diameter by 0.5 μ long, or as much as 1.5— μ long for the diplobacillary forms. The organism is similar to but more slender than the typhus rickettsia. It does not produce inclusion bodies. Experimental infection in guinea pigs results in solid immunity against subsequent infection.

*C. burneti* is indeed resistant to desiccation, disinfectants, sunlight, and to heat, surviving for several months in dried milk drops, placental debris and dust. Customary milk pasteurization temperature is about 75 percent effective. However, Enright *et al.* (4) reported that when vat holding temperature is raised to 145° F., the rickettsia in naturally infected milk is destroyed.

**Epidemiology**

Q fever has been reported from Australia, England, Africa, the United States, throughout Europe, Russia and the Near East. Recent surveys have shown that the disease is widely distributed in cattle throughout the United States. While cattle, sheep and goats appear to provide a natural reservoir for the infection, the disease is apparently asymptomatic in these animals, causing no observable pathology. That dairy cattle, sheep and goats are highly susceptible to natural infection, with rapid spread through the herds, is indicated by serological tests on blood and milk and isolation of the rickettsia.

The following animals have been found to be susceptible to experimental infection; field mouse, house mouse, porcupine, chipmunk, cottontail rabbit, and domestic cat. Serological evidence indicates natural infection in birds, notably sparrows, pigeons, parakeets, geese and in dogs.

The rickettsia has been recovered from no less than 15 species of ticks and further investigation may incriminate other biting insects.

Human susceptibility to *C. burneti* is attested to by a considerable number of reports of epidemics and isolated cases in Australia and continental United States.

**Transmission**

In the United States certain species of ticks and possibly other species of biting insects may transmit the disease. Parker and Davis (3) demonstrated transovarian infection in *Dermacentor andersoni* and others have demonstrated naturally occurring infection in this tick and in *D. occidentalis*, in the Lone Star tick, *Amblyomma americanum*, in the rabbit tick, *Hemaphysalis leporis palustris*, and in *Ixodes dentatus*. However, tick transmission of the disease appears to play no part in human infection in this country. Nevertheless, the feces of infected ticks are so rich in viable organisms that it is easy to believe that this enormous reservoir must play an important part in maintaining Q fever as an enzootic disease.

Of far more immediate importance is the fact that the fetal membranes, post-parturient vaginal discharges and milk of Q fever infected ruminants are highly contaminated with *C. burneti*, resulting in a corresponding contamina-
tion of the soil, water and roughage feed in and about dairy barns. Since this organism is highly resistant to desiccation and sunlight and since large dairies are frequently maintained close to or surrounded by housing developments in cities in the semi-arid sections of the southwest and far west, there is considerable opportunity for human infection.

Luoto et al. (5) found that parturient placentas of infected cows contained as many as 100,000,000 guinea pig infective doses per gram of tissue.

Welch et al. (6) reported that when the placental tissues of 72 sheep from an epidemic Q fever area in Northern California were examined for the presence of Coxiella burneti, the rickettsia was found with relative ease and with about the same frequency in both serologically positive and serologically negative animals. This has not been observed in serologically negative dairy cattle. Urine and feces are unlikely sources of infection but these reports indicate that placental tissues and vaginal discharges at the time of parturition are an important means of exit from the body of the infected animal.

Delay et al. (7) and Lennette et al. (8) recovered C. burneti from the air in and about cattle and goat dairies known to harbor infected animals. Rickettsia laden dust from infected dairies probably acts in the form of an aerosol to disseminate the infection to both animals and man in the immediate area and to the windward side. It is probable that the principal portal of entry is the respiratory tract. It appears reasonable to assume that the rarity of epidemics in man in central and eastern United States is due largely to the high rainfall, comparative freedom from dust storms, and differences in herd management.

Laboratory accidents may result in infection in laboratory employees. One such incident occurred in Montana (9). The role of contaminated milk as a transmitting agent via the digestive tract has not been definitely established. Although pasteurization of milk is only partially effective as a sterilization agent, the remaining viable organisms may be reduced in virulence and numbers to such extent that an infective dose rarely occurs. According to Luoto (10), approximately 50 percent of positive cows shed rickettsiae in their milk. Therefore, the ingestion of raw milk from infected herds may present a health hazard.

Q Fever in Animals

Q fever infection is being detected wherever a search for the disease is made. Shepherd (11) reported serological evidence of infection in beef and dairy cattle in 16 states in 1948. Subsequent surveys have indicated endemic infection in cattle in the Los Angeles area and infected sheep in northern California. Similar studies and surveys indicate cattle infection in Ohio, Wisconsin, Pennsylvania, Mississippi, Louisiana, North Carolina, Virginia, Texas, Iowa, New Jersey, Arizona, Nebraska, Georgia and Alabama.

As stated, the Q fever organism has a predilection for the reproductive organs of the pregnant female and for the mammary glands, with no observable systemic or pathological changes. However, Rostovtseva (12) reports a clinical syndrome of rhinitis, conjunctivitis, depression, anorexia, abortion
and decreased milk production in cows infected with Q fever in Russia. This may be due to strains of greater virulence than those prevalent in other countries.

**Q Fever in Man**

Q fever is an acute rickettsial disease of man. It is characterized by sudden onset, general malaise, fever, chills, severe sweats, retrobulbar headache, nausea and in about 50 percent of the cases patchy consolidation and mild lobar pneumonia. A dry cough and chest pain may develop by the fifth day. The incubation period varies between 14-25 days, although this period may vary apparently dependent upon the dose. Q fever is unique in that patients do not develop the typical rickettsial skin rash. Most cases have an uneventful recovery in two or three weeks although chronic cases have been reported. Unless complicated by secondary infection, few cases terminate fatally.

The disease may be readily confused with influenza, psittacosis, infectious hepatitis, non-icteric leptospirosis, brucellosis and primary atypical pneumonia.

Human Q fever has occurred in endemic and epidemic proportions in areas in California, Texas and Utah with isolated cases in Wisconsin, Ohio, Nebraska and just recently North Carolina. It has also been reported in other countries, notably Panama, England, Africa, Germany, Spain, Italy, Yugoslavia, Australia and Turkey.

Although it would appear to be eminently possible, tick to man or man to man transmission has not been definitely established. On the other hand, there is a high correlation between human infection and association with domestic ruminants and/or their products or living habitat within a contaminated dust fallout area.

According to Huebner (13), approximately 70-80 percent of the human cases in California could be related in some manner to livestock and 20-30 percent used raw milk in their households. All evidence indicates that man is an incidental host and that infection of man results almost exclusively from contact with infected animals, their products or their environment. Epidemiological evidence indicates that human infection, particularly in epidemic form, occurs by the respiratory route as the result of inhalation of contaminated dust or liquid particles. However, infection is relatively common in abattoir employees, indicating infection from direct contact or possibly by inhalation of contaminated water droplets.

Consumption of raw, massively contaminated dairy products or accidental direct contact with infected animals may be responsible for isolated cases. Despite the demonstration of small amounts of viable *C. burneti* in commercially pasteurized milk specimens, pasteurization has been shown to effectively reduce the occurrence of infection from dairy products.

It is difficult to explain the almost complete absence of clinical or serological evidence of human infection in the dairy employees and veterinarians in Georgia who have had close contact with known infected herds or among the random population living in proven infected areas in Georgia but the general
absence of contaminated dust and almost universal pasteurization of milk in eastern United States may be a major factor in the rarity of epidemics of Q fever in man in this section.

Approximately 5,000 human blood specimens submitted to the Georgia State Department of Public Health because of the existence of atypical pneumonia have been examined for Q fever. The complement fixation test is employed. Two human blood specimens reacted 1-4 with the complement fixation test but were negative to the Capillary Agglutination Test (CAT) test. One patient was the owner and the other the herdsman of a proven infected herd. We were unable to get second blood specimens. Thus, routine inclusion of the complement fixation test for Q fever in the battery of respiratory tests has revealed no evidence of widespread human infection in Georgia.

DIAGNOSIS

The diagnosis of Q fever is essentially a laboratory procedure. Individual or group infection of an influenza type, particularly following contact with ticks, ruminant animals or animal products, should be suspected. Paired blood samples should be examined for undulant fever, Q fever, leptospirosis, and influenza. Complement fixation procedures have been used but agglutination tests are, because of their simplicity, speed and validity, more applicable. Both the macroscopic tube and slide methods may be used. However, the capillary tube test developed by Luoto (14) promises to be the most acceptable method.

LABORATORY METHODS

Tjalma (15) made a statistical comparison study of the capillary agglutination and complement fixation tests for Q fever. He stated in summary that: (a) Except in one instance, no significant difference was found between the validity of the capillary agglutination and the complement fixation tests. In the case of the exception, the capillary agglutination test was found to be significantly more valid than the Iowa complement fixation test.

Stained C. burnetii antigen is used in the CAT method and only tiny amounts of sera or milk are necessary to complete the test. The capillary tube test is very sensitive and herds can be screened by testing vat milk. A screen test will be positive even though only one or two animals in the herd are reactors. In testing individual cows, composite samples of all lactating quarters should be collected.

Milk for testing should be shipped on wet ice to prevent souring. Whey has not been satisfactory in our experience. Hemolysis interferes with reading the test on blood, therefore, only sera should be sent to a laboratory.

Specimens of blood, milk or other media submitted for isolation of the organism should be frozen immediately after collection and shipped in dry ice. Isolation is made by animal inoculation.
Public health sanitarians routinely collect composite milk samples from all the commercial herds in the county which are carried or shipped in portable refrigerators to the State Department of Health Milk Laboratory for the customary tests for quality.

Screen CAT tests (1-1) were made on samples of milk collected during the period of study. All composite samples showing a screen titer of 1-1 were retested for maximum titer determination. Most of the herds were tested more than once. Individual cows in herds showing a positive titer of 1-64 or above were tested when practical.

To date surveys in Georgia have been confined largely to the Atlanta milk shed. Shipments of milk from south Georgia were sour on arrival and as implied above, CAT tests using whey were not satisfactory. Therefore, testing of milk shipped from distant areas was suspended.

There are very few flocks of sheep or goats in Georgia and these animals were not included in the survey.

A total of 1,167 dairy herds was tested for Q fever by the CAT method from 84 counties in Georgia, as shown in the accompanying map. There were four from South Carolina, one from Tennessee and two from Alabama. There were 146 reactor herds in 36 Georgia counties; one from South Carolina, one from Tennessee and two from Alabama.

Eight selected herds were examined by taking individual cow samples with the following results:

1. One herd with nonreactor composite sample—186 cows—all negative.
2. One herd with a 1-32 maximum titer composite sample—50 cows—six reactors.
3. Three herds with 1-64 maximum titer composite sample—209 cows—60 reactors.
4. Two herds with 1-128 maximum titer composite sample—116 cows—30 reactors.
5. One herd with 1-256 maximum titer composite sample—52 cows—25 reactors.

The herd listed in No. 1 above is a state-owned herd which was assembled in 1945, 1946 and 1947 by purchase of a small existing dairy and by purchase of heifers close to parturition date in New York, Wisconsin and Ohio over the three-year period. After 1947 there have been no additions to the herd except bulls. This herd is tuberculosis and brucellosis free.

Absence of serological evidence of infection in this herd is difficult to explain, since reacting herds were found in the same and adjoining counties. One is inclined to doubt that infection was present but died out with no serological evidence remaining. It is more probable that Q fever was not prevalent in the particular areas of origin at the time heifers were purchased and that the disease was not introduced. If this assumption is correct, it re-
emphasizes the importance of the dairymen raising their replacements except bulls.

* C. burneti* was isolated from milk from three herds all with a maximum titer of both composite sample and individual cows of 1-128 or above.
Table I shows the relative constancy of the titer of cows in one such herd. Considering that there is considerable change in cows tested due to cows going dry and fresh cows added in approximately eight months between the first and last test, the result is surprising. The cows changed but the number of animals in each category remained very much the same.

Ten herds showed increasing titer indicating recent infection and a susceptible cattle population.

Graph I shows a decided increase in titer of composite milk samples from three of these herds. This is considered to imply recent infection with consequent rapid increase in titer in a period of four months.

As shown in Graph II, the titer of 74 herds remained constant, give or take one tube throughout the 10-month study period. It would be difficult to imagine a more normal distribution of increasing, static and decreasing herd infection than that implied by Graph II.

A total of 656 human blood samples were tested by the CAT method with no reactors. These represent Battey State Tuberculosis Hospital patients, Red Cross mobile collections, practicing veterinarians and herdsmen intimately associated with proven infected herds or herds with positive titer indicative of active infection. With the exception of Battey Hospital patients, all human blood samples were obtained from people living in counties with known infected herds.

It seems clear then that there exists a wide prevalence of Q fever in cattle in North Georgia but that this situation is probably of long standing and is quite stable. There may therefore be little possibility of a flare-up of infection in a large number of animals at a given moment for there is as much immunity as disease. Whether this arises from a strain difference or from the circumstance that inocula are limited by rainfall and a general lack of dustiness can only be guessed at.

Owners, herdsmen and veterinarians who by the nature of their employment have every reasonable opportunity for infection may not be exposed in
Georgia to massive infection in cattle and this may explain the absence of infection in man. With zoonoses, perhaps it is necessary to produce an epizootic before an epidemic becomes possible.

CONTROL

The fact that *C. burneti* does not produce obvious pathological conditions in domestic animals nor influence general health or milk production in cattle is fortunate for our milk supply and the dairy industry. Moreover, this fact removes the economic necessity for the control or eradication of Q fever as is true with major infectious diseases such as brucellosis, tuberculosis and
CHANGES IN COMPOSITE MILK TITERS OF SEVENTY-FOUR CONSISTENTLY POSITIVE HERDS OVER A 10 MONTH PERIOD

GRAPH II

Titer Dilution Change

0 1 2 3 4

NUMBER OF HERDS

0 5 10 15 20

Tubes +4 +3 +2 +1 0 -1 -2 -3 -4
REVIEW OF Q FEVER IN GEORGIA

There is a tremendous volume of movement of cattle through sales barns and purchase of heifers for replacement in dairy herds which is difficult to regulate or control from the viewpoint of disease prevention. The data here presented indicate that such replacements should be held to the minimum practical level.

A satisfactory vaccine is not available for immunization of cattle, sheep, and goats. If it were available the expense incident to immunization of the bulk of cattle, sheep, and goats throughout the United States would be prohibitive. Since it is of little or no economic importance to husbandry, animal industry could not be expected to support such a program; neither could the individual farmer or dairyman.

State or federal regulations requiring testing and quarantine or disposal of infected animals and purchase of animals free of disease as is done with brucellosis and tuberculosis would be expensive and with our present knowledge would be impractical. Who would pay the cost of such a program?

Control of infection in man is based on prevention of spread from animals to man. In the presence of proven human epidemics in the vicinity of infected animal herds, the human population in exposure areas or persons subject to occupational hazard could be vaccinated, when and if a satisfactory vaccine is developed. This might apply to certain areas such as semi-arid areas in California and Texas where husbandry practices favor concentration of animals in close proximity to urban areas, thus facilitating the spread of the infection by aerosol. Vaccinations is neither indicated nor practical in the southeast where only an occasional case of human infection is reported.

It is reasonably safe to postulate that an immune phenomenon may occur in cattle which could be accelerated by vaccination.

A titer of 1-256 or higher is presumptive evidence of active infection in the herd and that part of the animals are shedding the virus in the milk, uterine fluids and placenta at time of parturition. How long the carrier state exists is unknown. A low titer, 1-8 or 1-16, is an indication that the individual animal or herd is or has been exposed to the infection. Repeated tests are necessary to determine the true status of the herd.

The consumption of raw milk is rapidly becoming a rarity. Pasteurization of milk is almost universal and is believed to be highly effective in controlling infection through ingestion of contaminated milk.

At the present time effective means for control of Q fever are not known. The medical profession and public health officials should be informed concerning the prevalence of the infection as an aid in diagnosis. Until adequate control measures are known, care should be taken in informing the general public because such information might result in fear of milk. Currently it appears that Q fever is a disease with which we will have to live. Fortunately for us in Georgia, it looks as if we are doing just that.

CONCLUSIONS

This study indicates that Q fever is widespread throughout the dairy herds of Georgia. There were no observable clinical symptoms of disease in any
of the herds studied nor in those persons who were already associated with
known infected animals. So far as we could determine, there was no alteration
in either the quality or volume of normal milk production in infected herds.

At the present time we do not have adequate information to justify
widespread control programs in cattle, sheep or goats. Discontinuance of
the practice of purchasing heifers close to parturition is fundamentally
sound disease control practice. It appears to be practical with respect to
Q fever as with other infectious diseases.

Although not available at present, vaccination may be practical in the
future under local conditions which would justify the expense involved as
a public health measure. Under conditions prevailing in Georgia at present,
undue agitation and a control program cannot be justified.

In view of the entire absence of observed or reported evidence of human
infection, the disease cannot be classified as one having public health
significance in Georgia. The results of this field survey appear to confirm
the impression gained from the continuing epidemiologic surveillance main-
tained on Q fever, psittacosis, and influenza.

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REPORT OF THE COMMITTEE ON PUBLIC HEALTH

Members of the Public Health Committee:


The importance of veterinary public health is gradually becoming more widely realized throughout the United States. A great deal of credit for this awareness is due to the fine work of veterinarians and official agencies actually engaged in this activity.

Since its beginning the United States Livestock Sanitary Association has been concerned with public health. The Constitution and By-Laws state, in part, that the purpose of the organization shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto. This purpose has been pursued in many fields of veterinary public health interest—tuberculosis, brucellosis, rabies, leptospirosis and anthrax, to mention just a few.

As our urban population expands, the need for public information programs on veterinary public health matters increases.

It is apparent that the pressures and problems of urbanization are not those of the city dweller alone but also greatly affect the farmer and livestock man. In previous times the majority of the people either actually lived on farms or had a farm background. This is no longer true. Those individuals familiar with farm problems are in the minority. The remainder have little or no comprehension of these difficulties. This ignorance is reflected in many fields but is of particular importance in the formation of legislation on local, state and national levels. Some of these laws relate to animal health with reference to effect upon the human being. Too often legislation is passed which is of little value in the protection of public health and may be a detriment to the livestock industry. Under the guise of public health urgency the lawmakers are sometimes stampeded to pass measures of this kind. They do so because of lack of correct information. It is our duty to see that legislators are informed on matters of veterinary public health.

ANTIBIOTICS IN FLUID MILK

The 1959 report of the Public Health Committee contained the results of four surveys on antibiotics in fluid milk conducted and reported by the Food and Drug Administration, United States Department of Health, Education and Welfare (1). The 1959 survey indicated a significant drop in the per-
percentage of contaminated samples compared to the previous figures. This was encouraging. However, the problem has not been completely solved.

It is evident that despite the intensive educational campaign, many dairy farmers and some veterinarians using antibiotics in the treatment of animal diseases—particularly mastitis—are uninformed concerning the elimination of antibiotics in milk. Therefore, many states have formulated available material into recommendations.

Generally, these recommendations are as follows (2):

I. Udder Infusions
   a. Penicillin—strictly aqueous base— withhold milk from all quarters at least 72 hours.
   b. Penicillin—strictly oil base—withhold milk from all quarters at least eight days.
   c. Penicillin—combined aqueous and oil base—withhold milk eight days or according to label instructions.
   d. Terramycin or Aureomycin—aqueous or oil—withhold milk from all quarters for at least six days.

II. Untreated quarters—Antibiotics can appear in untreated quarters of an udder after one or more quarters are treated. Therefore, when one quarter is treated, the milk from all quarters must be withheld.

III. Intramuscular or intravenous injections—Milk should be withheld a minimum of five days or longer depending upon circumstances.

IV. Feed supplements—Antibiotic-containing feeds are not recommended for dairy cows.

At the present time regulations are being adopted by the Food and Drug Administration which may change these recommendations considerably.

It must, of course, be realized that the suggestions may not apply to all preparations and conditions and must be adapted to the situation.

CHEMICAL FLY CONTROL ON DAIRIES

In view of the problem of insecticides in milk, the Public Health Committee considers it advisable to set forth general recommendations concerning their use on dairies (3).
## 1960 CHEMICAL FLY CONTROL ON DAIRIES

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Formulation</th>
<th>Amount</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual sprays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside and outside barn</td>
<td>Diazinon 25% emulsion concentrate</td>
<td>1 to 2 gallons plus water to make 50 gallons of spray</td>
<td>Can be used with or without sugar (10 to 25 lbs.). Spray interior and exterior surfaces to point of runoff. Do not use in milk rooms. Avoid contamination of feed and water. Do not apply to animals. Thoroughly flush mangers and watering troughs after application. Agitation necessary to keep wettable powder suspended in spray mixture.</td>
</tr>
<tr>
<td></td>
<td>Diazinon 25% wettable powder</td>
<td>8 to 16 pounds plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td></td>
<td>Malathion 55 to 57% emulsion concentrate</td>
<td>1 to 1½ gal. plus water to make 50 gal. of spray</td>
<td>Not effective in areas where resistant house flies have developed.</td>
</tr>
<tr>
<td></td>
<td>Malathion 25% wettable powder</td>
<td>20 pounds plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td></td>
<td>Korlan 12% emulsion concentrate</td>
<td>4½ gal. plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td></td>
<td>Korlan 25% wettable powder</td>
<td>16 pounds plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td></td>
<td>Methoxychlor 25% emulsion concentrate</td>
<td>5 gal. plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td></td>
<td>Methoxychlor 50% wettable powder</td>
<td>16 pounds plus water to make 50 gal. of spray</td>
<td>Same as above.</td>
</tr>
<tr>
<td>Dry baits</td>
<td>Commerically prepared baits</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Dipterex (Dylox)</td>
<td>Use as prepared by manufacturer</td>
<td>Apply 2 to 4 ozs. per 1,000 sq. ft. on floors, window sills, and other areas where flies congregate. Apply daily until fly population is reduced, then apply once or twice a week as necessary. Do not contaminate feed, mangers, water or watering troughs.</td>
</tr>
<tr>
<td></td>
<td>DDVP</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Diazinon</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malathion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator prepared baits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diazinon 25% wettable powder</td>
<td>1 pound to 24 pounds of granulated sugar</td>
<td>Same as commercially prepared baits.</td>
</tr>
<tr>
<td></td>
<td>Malathion 25% wettable powder</td>
<td>2 pounds to 23 pounds of granulated sugar</td>
<td></td>
</tr>
<tr>
<td><strong>Liquid baits</strong></td>
<td><strong>Dipterex (Dylox) commercial bait</strong></td>
<td>1 pound to 4 gal. water plus 4 pounds sugar</td>
<td>Use 1 to 3 gal. per 1,000 sq. ft. on floors, window sills and other places where flies congregate. Apply daily until the population is reduced then apply once or twice a week as necessary. Do not contaminate feed, mangers, water or watering troughs. If surface is of dirt or debris, apply bait on sheets of metal, wood, paper, gunny sacks, etc.</td>
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<tr>
<td></td>
<td>Diazinon 25% emulsion concentrate</td>
<td>4 fluid ounces plus 1 pound sugar to 5 gal. water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malathion 55% emulsion concentrate</td>
<td>Same as above</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Korlan 25% wettable powder</td>
<td>2 pounds plus ½ pound sugar to 3 gal. water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DDVP 10.3% emulsion concentrate</td>
<td>6½ fluid ozs. plus 4 to 5 pounds sugar and water to make 5 gal.</td>
<td></td>
</tr>
<tr>
<td><strong>Insecticide treated fly cords</strong></td>
<td></td>
<td></td>
<td>Same as before.</td>
</tr>
<tr>
<td></td>
<td>Parathion plus Diazinon commercially treated cords</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Space sprays</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyrethrins or Allethrin plus synergists, thiocyanates, and other proprietary mixtures</td>
<td>As directed by manufacturer</td>
<td>Spray directly on resting flies or fog in air where flies are numerous. No residual effect on flies entering premises after treatment. Time applications to provide protection during periods of maximum barn use, and maximum fly activity.</td>
</tr>
<tr>
<td><strong>Sprays on Cattle</strong></td>
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<tr>
<td></td>
<td>Emulsion concentrate</td>
<td>Dilute with water as directed by manufacturer</td>
<td>Spray entire body at rate of 2 quarts per animal. Will kill flies on contact and repel flies from landing on animals after treatment. Also effective for use with automatic treadle sprayers as directed by manufacturer.</td>
</tr>
<tr>
<td></td>
<td>Pyrethrins or Allethrin with piperonyl butoxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oilbase</td>
<td>Ready for use as directed by manufacturer</td>
<td>Fog or fine mist-spray over entire animal wetting outer hair only. Two to 3 ounces maximum per animal, as directed by manufacturer. Also effective for use with automatic treadle sprayers.</td>
</tr>
<tr>
<td></td>
<td>Pyrethrins or Allethrin with piperonyl butoxide</td>
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<td></td>
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<tr>
<td><strong>Dust on Cattle</strong></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Methoxychlor 50% wettable powder</td>
<td></td>
<td>For control of horn flies sprinkle one heaping tablespoonful on poll and neck; rub in lightly by hand. Repeat at 3-week intervals as long as necessary.</td>
</tr>
<tr>
<td><strong>Larvicides</strong></td>
<td></td>
<td></td>
<td>Apply as a coarse spray or with a sprinkling can at a rate of 5 gal. spray per 500 sq. ft. of manure piles. Repeat as additional manure is added.</td>
</tr>
<tr>
<td></td>
<td>Diazinon 25% emulsion concentrate</td>
<td>4 fluid ounces plus 5 gal. water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malathion 55% emulsion concentrate</td>
<td>10 fluid ounces plus 5 gal. water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Korlan 25% wettable powder</td>
<td>1.5 to 2.5 pounds plus 5 gal. of water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DDVP 10.3% emulsion concentrate</td>
<td>6½ fluid ozs. plus water to make 5 gallons</td>
<td></td>
</tr>
</tbody>
</table>
Here again these are recommendations only and the use of the insecticides must be in conformity with local requirements.

As this report is being written many tests on the use of insecticides are under way and undoubtedly will result in changes and additions during the coming year.

**TUBERCULOSIS**

In those areas of the world where the incidence of tuberculosis in dairy cattle is very low the problem of non-specific sensitivity to mammalian tuberculin is being observed. Berman, *et al.*, reported their investigations in Wisconsin at the United States Livestock Sanitary Association meeting in 1959 (4).

Johnson (5) further explored the subject of non-specific sensitivity at the 1960 convention of the American Veterinary Medical Association.

European investigators are presently engaged in very comprehensive studies of the subject, particularly in Germany, Czechoslovakia, and the United Kingdom (6).

Non-specific sensitivity is probably one of the major road blocks in the path of eradication of bovine tuberculosis.

The Public Health Committee is very much encouraged by the renewed interest in tuberculin test research. We recommend that this Association vigorously support the efforts being made on non-specific sensitivity to mammalian tuberculin.

**MANDATORY POULTRY INSPECTION**

The Poultry Products Inspection Act which became effective January 1, 1959, is administered by the Poultry Division, Agricultural Marketing Service of the United States Department of Agriculture. All processed poultry and poultry products intended for interstate or foreign shipment must be inspected.

Eight hundred twenty-one plans are operating under the federal inspection program. During the first year of operation approximately 4.75 billion pounds of poultry were inspected and certified as wholesome. In frying chickens, the principal causes of condemnation were: septicemia, cadavers, tumors, parasites, and decomposition (7).

The United States Livestock Sanitary Association has been very active in recommending the development of regulations governing the inspection of poultry and poultry products. The Public Health Committee is gratified with the progress made by the federal government in this direction.

We recommend that each state and territory adopt similar poultry inspection regulations for the protection of the public health within its jurisdiction.

**RECOMMENDATIONS**

1. Increased activity in public information on veterinary public health with particular reference to those matters which may result in legislation.
2. Vigorous support of research on non-specific sensitivity to mammalian tuberculin.

3. Adoption of mandatory poultry inspection regulations by states and territories, utilizing the federal regulations as representing the national standards.

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INTRODUCTION

Great progress has been made in the control of rabies in the United States during the past fifteen years. In 1946 there were more than 10,872 cases of animal rabies reported, 8,384 of which were in dogs. The number of reported cases has declined steadily until in 1959 there was a total of 4,083 cases of animal rabies and just 1,119 of these occurred in dogs. These great strides in the control of rabies are a reflection of the concerted research directed toward the development and improvement of canine rabies vaccine and the establishment of sound rabies control programs in health departments throughout the country.

In contrast to this measure of success which has been achieved with the control of the disease in dogs, wildlife rabies has been on the increase during this same period. Thus, where dog rabies cases have declined 87 percent during the past 15 years, the number of wildlife rabies cases has doubled—in spite of obviously poorer reporting of wild animal diseases. In 1958 and 1959 the number of reported wild animal rabies cases surpassed the number of cases in dogs for the first time on record. Thus, it has become obvious that future research must be directed toward the wildlife rabies problem if this disease is to be ultimately controlled or possibly eventually eradicated.

In the United States there are discrete areas where the fox and skunk are the apparent primary wildlife vectors. Within many of the states where only fox rabies is reported, there are high populations of skunks with little or no skunk rabies cases. The opposite is often true in those regions where only skunk rabies is reported.

COMPARATIVE INFECTIVITY STUDIES

We should like to report here on one phase of investigations at the CDC Southeast Rabies Station which was designed to gain information about the behavior of rabies in the species of wildlife indigenous to the southern states. The first study was a comparison of the infection in foxes and skunks, followed by the same design in raccoons and opossums.

In these studies we chose a fox salivary gland rabies virus isolated from a county in Alabama which had experienced an epizootic of fox rabies. We inoculated tenfold dilutions of this virus into groups of six to seven animals per group of each species. In the first study a total of 28 foxes and 25 striped skunks were inoculated and studied simultaneously. In the second study 35
raccoons and 17 opossums were inoculated. All animals were inoculated in the cervical muscles except three opossums; these were inoculated intracerebrally. A complete description of these and other studies has been reported elsewhere and for purposes of this presentation, we should like to give the most significant results including a comparison of the infection in four species of wild animals and our epidemiological interpretation in these findings.

As shown in Table 1, 24 of the 26 foxes died of rabies when inoculated with doses ranging from 14 to 14,000 mouse LD50s (MLD50s). The disease in foxes which received the three highest doses varied in incubation period from 12 to 24 days, while those in the group which received only 14 MLD50s had incubation periods varying from 23 to 109 days. Seventeen of the 24 rabid foxes demonstrated signs of furious (excitative phase) rabies; the other seven had paralytic (dumb) rabies.

**TABLE 1**

*Comparative Results of Inoculation of Street Rabies Virus in Foxes and Skunks*

<table>
<thead>
<tr>
<th>Inoculum (MLD50)</th>
<th>Rabies Deaths</th>
<th>Incubation Period (Days)</th>
<th>Period of Clinical Illness (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Inoculated</td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>Foxes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14,000</td>
<td>6/7</td>
<td>12-17</td>
<td>13</td>
</tr>
<tr>
<td>1,400</td>
<td>7/7</td>
<td>15-18</td>
<td>16</td>
</tr>
<tr>
<td>140</td>
<td>4/5*</td>
<td>16-24</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>7/7</td>
<td>23-109</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>24/26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skunks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140,000</td>
<td>7/7</td>
<td>17-78</td>
<td>22</td>
</tr>
<tr>
<td>14,000</td>
<td>6/6</td>
<td>14-20</td>
<td>19</td>
</tr>
<tr>
<td>1,400</td>
<td>5/6</td>
<td>17-88</td>
<td>76</td>
</tr>
<tr>
<td>140</td>
<td>0/4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18/23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Excluding two deaths from other causes.

Eighteen of 23 skunks died of rabies; none of those which were inoculated with 140 MLD50s died of rabies. The incubation period in the skunks varied from 17 to 88 days. Twelve of the 18 rabid skunks displayed the excitative (furious) stage of the disease and in six of these the signs lasted longer than four days. None of the 24 rabid foxes showed clinical signs lasting longer than three days.

The same comparison is available for raccoons and opossums in Table 2. The outstanding features demonstrated in these data are: (1) no opossums inoculated intramuscularly died of rabies and (2) although two raccoons inoculated with only 160 MLD50s died of rabies, not one of the various concentrations of virus killed all of the raccoons in contrast with the foxes and skunks.
TABLE 2

Comparative Results of Inoculation of Street Rabies Virus in Raccoons and Opossums

<table>
<thead>
<tr>
<th>Inoculum (MLD₅₀)</th>
<th>Rabies Deaths</th>
<th>Incubation Period (Days)</th>
<th>Period of Clinical Illness (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Inoculated</td>
<td>Range Median</td>
<td>Range Median</td>
</tr>
<tr>
<td>Raccoons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,000</td>
<td>5/7</td>
<td>13-35 19</td>
<td>1.13 5.0</td>
</tr>
<tr>
<td>1,600</td>
<td>4/7</td>
<td>17-42 26</td>
<td>3.7 4.0</td>
</tr>
<tr>
<td>160</td>
<td>2/4*</td>
<td>10-13 12</td>
<td>&lt;1-3 2.0</td>
</tr>
<tr>
<td>16</td>
<td>0/3†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>0/3†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11/24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opossums</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80,000</td>
<td>0/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,000 (IM)</td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,000 (IC)</td>
<td>1/3</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1/19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Excluding three deaths from other causes.
† Excluding four deaths from other causes.

From these results, it was possible to calculate the 50 percent mortality endpoint of the strain of fox salivary gland rabies virus used for infecting these four species of wildlife. In each of the experiments simultaneous titrations were conducted in mice which served as a standard comparison. Titers in each of these animals is shown in Table 3. Although the number of animals per group are too small to determine the actual threshold of infection for each species, we can calculate the dose which is required to kill 50 percent of the animals of each of these species. These results are listed in Table 4; they are obtained by subtracting the logarithmic 50 percent mortality endpoint (titer) in each species from the mouse intracerebral titer, then converting the logarithmic function back to its arithmetic value, giving a dosage value expressed in MLD50s.

TABLE 3

*Titration Values of a Street Rabies Virus Isolant in Various Species of Animals*

<table>
<thead>
<tr>
<th>Exp. No. 1</th>
<th>3-Week Mice Intracerebral</th>
<th>4-Week Hamsters Intramuscular</th>
<th>Foxes Intramuscular</th>
<th>Skunks Intramuscular</th>
<th>Raccoons Intramuscular</th>
<th>Opossums Intramuscular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. No. 2</td>
<td>7.1</td>
<td>4.1</td>
<td>&gt;6.4</td>
<td>4.4</td>
<td>3.2</td>
<td>&lt;1.3</td>
</tr>
</tbody>
</table>

* Titers expressed as reciprocal of log LD₅₀ per 0.6 ml. inoculum.

Having thus observed differences in the susceptibility of these groups of wild animals to this street rabies virus isolant, the next step was to determine
whether any differences existed in the emission of rabies virus in the saliva of the infected animals; these data were related to the amount of virus necessary to infect each animal.

Beginning on the 10th day after inoculation, each animal (fox, skunk, coon or opossum) was removed from its cage, placed on a specially designed table and a saliva sample was collected by withdrawal into a syringe or by a cotton tipped applicator swab or both. At least two series of samples were thus obtained each week and if the animal displayed rabies symptoms, more frequent samples were collected. Results of this phase of the work are shown in Table 5.

### Table 5

**Data on Demonstration of Rabies Virus in Saliva of Wild Animals Experimentally Injected by the Intramuscular Route**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Foxes</th>
<th>Skunks</th>
<th>Raccoons</th>
<th>Opossums</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. with Virus in Saliva</strong></td>
<td>10/24 (41%)</td>
<td>15/18 (83%)</td>
<td>7/11 (63%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>No. Died of Rabies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. with at least 1,000 MLD&lt;sub&gt;50&lt;/sub&gt; Virus in Saliva</strong></td>
<td>2/10 (20%)</td>
<td>11/15 (73%)</td>
<td>2/7 (28%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>No. with Virus in Saliva</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. Succumbing to &gt;10,000 MLD&lt;sub&gt;50&lt;/sub&gt; of Challenge Virus</strong></td>
<td>1/6 (16%)</td>
<td>11/13 (84%)</td>
<td>3/5 (60%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>No. with Virus in Saliva</strong></td>
<td>8/11 (73%)</td>
<td>0</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td><strong>No. Succumbing to &lt;1,000 MLD&lt;sub&gt;50&lt;/sub&gt; of Challenge Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Higher percentages of infected submaxillary salivary glands were obtained in the skunks and raccoons than in the foxes. Since saliva specimens were not collected daily, certainly some animals conceivably could have had virus-positive salivas during the intervals that specimens were not collected. However, in the group of foxes which were inoculated with the most virus, saliva samples were collected five or six, two and zero days before death. Only one of these heavily-dosed animals yielded virus in its saliva. Even though five animals in this group had virus-positive salivary glands, only one of these contained enough virus to be detected in the saliva sample. Of the seven
rabid foxes infected with the lowest challenge dose (14 MLD50), six yielded virus-positive saliva specimens.

The next major point of interest was that the saliva of skunks contained consistently more virus per 0.03 ml than did the foxes or raccoons. Samples from eight of the 15 saliva-positive skunks contained greater than 20,000 MLD50s of virus per 0.03 ml, and three of these yielded almost 1,000,000 MLD50s per 0.03 ml. The saliva of only two foxes contained greater than 1,000 MLD50s per 0.03 ml.

DISCUSSION

Although the number of inoculated animals in each of the groups inoculated with the various dilutions is not large enough to give close confidence limits for ecological generalizations, there are obvious quantitative differences among the four host species studied in their pathogenetic reactions to the single rabies virus isolant with which they were inoculated. This may partially explain the discretely demarcated geographic distribution of fox and skunk rabies in the United States. Foxes infected with large doses of rabies virus generally would not be expected to have infective saliva. If they succumb to the infection, it is usually after a very short incubation period of approximately two weeks which is not sufficient time to allow salivary glands and hence saliva, to become adequately infected. On the other hand, if foxes are infected with smaller doses (10 to 1,000 MLD50s) it is conceivable for a majority of them to develop infective saliva which helps to perpetuate the disease among foxes. Our experiments have shown that a greater dose of this southeastern fox rabies virus isolant is required to infect skunks. It is probable that only a small percentage of the infected foxes emit enough virus to infect skunks. In the southeastern states there might be such a strain of virus at work which limits the transmission of the disease to foxes. It is conceivable, however, that with quantitative host-virus adaptive changes in the future we might expect to encounter more skunk rabies in the southeastern states if the incidence of fox rabies remains high and a great enough number of skunks are exposed. The opposite is probably true of the midwest where skunk rabies predominates.

Many other factors, such as differences in the invasiveness of virus strains and extrinsic ecological influences obviously play a role in this species-discrete geographical distribution of wildlife rabies. We need to know more specifically what differences occur in these areas which will help us understand the natural history of this disease. Such information is essential before scientifically effective methods for controlling sylvatic rabies can be devised.
REPORT OF THE COMMITTEE ON RABIES


Rabies Incidence and Trends—Calendar Year 1959

The total number of laboratory confirmed animal rabies cases reported for calendar year 1959 was 4,083, representing a decrease of 731 cases from the total number of confirmed cases reported during the previous calendar year. By the same token, the number of cases in dogs declined from 1,643 to 1,119, a decrease of 524. Thus the decrease in dog rabies accounted for 72 percent of the decline in the total incidence of animal rabies in the United States from calendar year 1958 to calendar year 1959. This is indeed a heartening trend in the face of the robust increase of the dog population to over 26 million. Over the entire country, an estimated seven million dogs were vaccinated against rabies last year.

The geographic distribution of rabies in the United States was substantially the same as that for the previous year. (Figure 1—Map.) The Pacific Northwest and the Upper Rocky Mountain states remained rabies-free except for six cases in bats in Montana which were found positive in surveys conducted by the United States Public Health Service laboratory at Hamilton, Montana. Newly infected areas were uncovered in Colorado, with 16 cases being reported in that state during 1959. The New England states also remained rabies-free, except for a single rabid bat reported in Connecticut and a puzzling case in a horse in Maine. The State of Hawaii remains rabies-free.

Substantial drops in rabies incidence occurred in Indiana, Ohio, Wisconsin, Minnesota, Kentucky, Virginia, West Virginia, South Carolina, Georgia and Mississippi. States which experienced increased incidence in total rabies cases over the previous year were New York, Texas, Missouri, Arkansas, Alaska and the Commonwealth of Puerto Rico.

The biggest increase in incidence was in New York state which reported 478 cases for 1959 as compared to 261 cases during 1958. This increase is the result of an epizootic of fox rabies in seven counties of previously rabies-free western New York state and represents the worst outbreak in the state since 1946.

Human Rabies Deaths—1959

Table 1 gives epidemiological data on the seven human rabies deaths which occurred in the United States during calendar year 1959. Perhaps the most striking fact shown is that two of the seven cases were attributed to exposure by bats. In fact, in less than a 12-months period (11/4/58-9/3/59), three cases of human rabies in the United States were attributed to bats. In two of the 1959 cases the exposure was from owned, unvaccinated rabid dogs.

273
FIGURE 1
RABIES—REPORTED LABORATORY CONFIRMED CASES
U.S., BY STATE, 1959

Total - 4,083

Source: USDA, ADE Division Notice
March 1960 and CDC data
<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Los Angeles County, California</td>
<td>6/3/59</td>
<td>54</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>9 days</td>
<td>None</td>
<td>Unknown—Probably bats in Texas caves</td>
</tr>
<tr>
<td>2. Birmingham, Jefferson County,</td>
<td>6/8/59</td>
<td>30</td>
<td>F</td>
<td>Bite on finger</td>
<td>Approx. 86 days</td>
<td>15 days</td>
<td>None</td>
<td>Neighbor's dog</td>
</tr>
<tr>
<td>3. Sullivan County, Indiana</td>
<td>7/7/59</td>
<td>4</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>8 days</td>
<td>None</td>
<td>Unknown—Probably dog</td>
</tr>
<tr>
<td>4. Richland County, Wisconsin</td>
<td>9/3/59</td>
<td>44</td>
<td>M</td>
<td>Bite on ear lobe</td>
<td>22 days</td>
<td>6 days</td>
<td>None</td>
<td>Bat—not examined</td>
</tr>
<tr>
<td>5. Atlanta, Fulton County, Georgia</td>
<td>10-1-59</td>
<td>10</td>
<td>M</td>
<td>Bite on left arm above elbow</td>
<td>40 days</td>
<td>3 days</td>
<td>21 doses vaccine</td>
<td>Dog—confirmed rabid</td>
</tr>
<tr>
<td>6. Ft. Leonard, Wood, Missouri</td>
<td>11/14/59</td>
<td>23</td>
<td>M</td>
<td>Unknown</td>
<td>Unknown</td>
<td>2 days</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>7. Florence, South Carolina</td>
<td>12/14/59</td>
<td>65</td>
<td>M</td>
<td>Face bites</td>
<td>21 days</td>
<td>3 days</td>
<td>None</td>
<td>Stray dog</td>
</tr>
</tbody>
</table>
It will be noted that in only one of the seven human rabies cases reported in 1959 was post exposure immuno-prophylactic treatment given; this was in a 10-year-old boy in Atlanta, Georgia, bitten by a confirmed rabid dog. The dog in the case was the last rabid animal reported in Atlanta in 1959. In two of the seven cases reported, the victims were under 15 years of age. The incubation periods of the four known cases were 21, 22, 40 and 86 days respectively. The case in Birmingham, Alabama, had a rather long (15 days) clinical course.

**TABLE 2**

*Rabies in Animals*

First Nine Months—1959-1960

(NoVS—United States Public Health Service)

<table>
<thead>
<tr>
<th>State</th>
<th>1959 (First 9 months)</th>
<th>1960 (First 9 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New England</strong></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>0</td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td>0</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><strong>Middle Atlantic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>276</td>
<td>393</td>
</tr>
<tr>
<td>New Jersey</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><strong>East North Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>65</td>
<td>95</td>
</tr>
<tr>
<td>Indiana</td>
<td>95</td>
<td>73</td>
</tr>
<tr>
<td>Illinois</td>
<td>12</td>
<td>43</td>
</tr>
<tr>
<td>Michigan</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>78</td>
<td>19</td>
</tr>
<tr>
<td><strong>West North Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>211</td>
<td>92</td>
</tr>
<tr>
<td>Iowa</td>
<td>149</td>
<td>133</td>
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<td>Missouri</td>
<td>139</td>
<td>193</td>
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<td>North Dakota</td>
<td>47</td>
<td>34</td>
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<td>South Dakota</td>
<td>90</td>
<td>48</td>
</tr>
<tr>
<td>Nebraska</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>Kansas</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td><strong>South Atlantic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delaware</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maryland</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>District of Columbia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Virginia</td>
<td>127</td>
<td>177</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>2,851</td>
</tr>
<tr>
<td><strong>East South Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>180</td>
<td>101</td>
</tr>
<tr>
<td>Tennessee</td>
<td>102</td>
<td>145</td>
</tr>
<tr>
<td>Alabama</td>
<td>170</td>
<td>59</td>
</tr>
<tr>
<td>Mississippi</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>West South Central</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td>201</td>
<td>264</td>
</tr>
<tr>
<td>Louisiana</td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Texas</td>
<td>349</td>
<td>406</td>
</tr>
<tr>
<td><strong>Mountain</strong></td>
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<tr>
<td>Montana</td>
<td>0</td>
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<td>Idaho</td>
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</tr>
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<td>Wyoming</td>
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<td>0</td>
</tr>
<tr>
<td>Colorado</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>New Mexico</td>
<td>6</td>
<td>15</td>
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<td>Arizona</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Utah</td>
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</tr>
<tr>
<td>Nevada</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Pacific</strong></td>
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<td></td>
</tr>
<tr>
<td>Washington</td>
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</tr>
<tr>
<td>Oregon</td>
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<td>0</td>
</tr>
<tr>
<td>California</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>2,728</td>
</tr>
</tbody>
</table>
RABIES

National Rabies Picture—First Nine Months—1960

The total number of animal rabies cases during the first nine months of 1960 is 2,728 (Table 2). These are provisional figures and are based on cumulative totals reported on a weekly basis to NOVS, United States Public Health Service. This represents a decrease of 123 cases as compared with the same period in 1959. In this period a rise in incidence was noted to continue in Missouri, Arkansas, Texas and New York State. New increases were noted in Virginia, West Virginia, and Colorado, and notable declines in incidence were reported from Minnesota, Iowa, South Dakota, North Carolina, Georgia, Florida, Kentucky, and Alabama.

Local areas of high incidence thus far during 1960 have been in foxes in the Brazos River Valley of east-central Texas, dog rabies in the Brownville-Matamoros Border area and an explosive outbreak of canine rabies in the Calexico-Mexicali area of California—Baja, California.

Human Rabies Deaths—1960 to Date

At this writing (October 19, 1960) there is every indication that human rabies deaths in the United States will have reached an all-time low by the end of calendar year 1960 (Table 3). The only indigenous case of human rabies reported thus far for this year was in a nine-year-old boy in Atlanta, Georgia, who died on May 21, 1960. This was a particularly sad case complicated by mistaken identity of the biting dog. It seems that the wrong dog was incarcerated for clinical observation and immunoprophylactic treatment of the victim—who was one of seven bitten—was stopped after he had received hyperimmune serum and seven daily inoculations of vaccine. He received multiple bites on the nose, lip, elbow and back, and the incubation period in this case was 56 days.

The only other human rabies death occurred in Cleveland, Ohio, in a 19-year-old girl who was bitten by a cat during a visit to Guatamala City, Guatamala. The cat was later proved rabid, but the biting incident was not reported to the public health authorities in Guatamala and there was no treatment administered.

Bat Rabies

During 1959 five new states were added to the list of states reporting bat rabies, which brought the total up to 25 states reporting the disease in bats up to the end of the calendar year. These states were West Virginia, Connecticut, Virginia, Illinois, and Maryland.

Thus far in 1960, five additional states have reported bat rabies: Oregon, Indiana, Missouri, Iowa and New Jersey. This now makes a total of 30 states reporting bat rabies since the first reported case in Florida in 1953 (Figure 2). It is interesting to note that bat rabies has been reported from
<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Atlanta, Fulton County, Georgia</td>
<td>5/21/60</td>
<td>9</td>
<td>M</td>
<td>Bitten through nose and lip, elbow and lower back</td>
<td>56 days</td>
<td>3 days</td>
<td>Serum + 7 doses vaccine</td>
<td>Dog or dogs running amuck</td>
</tr>
<tr>
<td>2. Guatemala City, Guatemala</td>
<td>9/5/60</td>
<td>19</td>
<td>F</td>
<td>Nip on the right hand</td>
<td>50 days</td>
<td>5 days</td>
<td>None</td>
<td>Cat</td>
</tr>
</tbody>
</table>
four states which have been consistently free of rabies in terrestrial animals over the years. These are Oregon, Connecticut, Montana, and New Jersey.

The record now contains five human rabies deaths in the United States which have been attributed to rabid bats (Table 4). Epidemiological and virological investigations on rabies in bats and other wild animal vectors are continuing in the three regional field stations of the Communicable Disease Center.

Pre-exposure Prophylaxis

The latest information on studies carried out in pre-exposure immunization of high-risk groups of persons indicates that good antibody response can be expected following a schedule consisting of a primary series of three weekly inoculations of avian origin rabies vaccine followed by a booster of the same does four to six months following the primary series. Recent studies have indicated that the duck-embryo vaccine elicits a positive serum neutralizing antibody response in a greater percentage of individuals than does the HEP chick-embryo vaccine.
<table>
<thead>
<tr>
<th>Year</th>
<th>Locality</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Length of Illness</th>
<th>Incubation Period</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>Big Spring, Texas</td>
<td>41</td>
<td>F</td>
<td>Bite, hands, forearm</td>
<td>8 days</td>
<td>16 days</td>
<td>None</td>
</tr>
<tr>
<td>1956</td>
<td>Austin, Texas</td>
<td>41</td>
<td>M</td>
<td>Unknown, worked with rabid bats</td>
<td>3 days</td>
<td>Unknown</td>
<td>None</td>
</tr>
<tr>
<td>1958</td>
<td>Butte County, California</td>
<td>53</td>
<td>F</td>
<td>2 bites on fingers</td>
<td>11 days</td>
<td>55 days</td>
<td>Serum, systemically, 14 doses vaccine 4 days after bite</td>
</tr>
<tr>
<td>1959</td>
<td>Los Angeles County, California</td>
<td>54</td>
<td>M</td>
<td>Unknown, probably bats in Texas caves</td>
<td>9 days</td>
<td>Unknown</td>
<td>None</td>
</tr>
<tr>
<td>1959</td>
<td>Richland County, Wisconsin</td>
<td>44</td>
<td>M</td>
<td>Bite on ear lobe</td>
<td>6 days</td>
<td>22 days</td>
<td>None</td>
</tr>
</tbody>
</table>
When animals now domesticated were wild and lived at random, in a sense, there were no problems. No one was greatly concerned. Problems were recognized when someone began to flavor nature with his will in order to attain certain goals. As time passed, the animal industry as we know it became a highly systematized effort to provide the economy with a wide variety of products derived from animals. Just as complex and interrelated as the industry itself, are the disease problems it faces from day to day. As the objectives, and the methods of achieving them shift, so do the problems faced. The shape of what can be accomplished in harnessing nature is influenced by what is sought and that which is possible with the means at hand. That which is possible is in turn partially a product of our ability to utilize natural forces to advantage, recognizing that what may not even be possible today may be commonplace tomorrow. In short, we have much to do with deciding what our problems are.

One objective shared by nearly all those in the swine industry is to enjoy a profit from his efforts. The garbage feeder, the pure bred swine raiser, the commercial pork producer, the packer, the sale barn operator and the rest all conduct their activities with an eye to making a profit. This has a great influence on thoughts about just what constitutes a disease problem, how things will be accomplished, what things will be done, and even who will do them.

The problems of financing, type of enterprise, marketing, and husbandry all have an impact on the problems of disease; just as the converse is true. The margin of profit a producer anticipates may dictate whether his swine will be immunized against a disease and/or whether some physical improvement will be made to better house them. If the enterprise consists of buying weaned pigs to fatten for market then transmissible gastroenteritis is of little consequence to him. The shipper and the packer are principally concerned with bruises and puncture wounds. Even so, it does not take much imagination to see how economic forces influence the nature and extent of causes for concern about disease.

Each enterprise in each recognizable unit of the industry is physically a little different from the rest; each one is operated a little or a lot differently from others. These things also have an impact on what the problems are and the weight of them. To compound this complexity, nature has provided a remarkable maze of variables in the raw material—pigs. When the combinations and permutations of these variables are considered the possibilities reach prodigious proportions.
At nearly all the meetings of those interested in swine diseases the comment is made that our reporting systems are inadequate. Most everyone will agree that the best we have is none too good. It is interesting, however, how well we are able to recognize the greatest and the least of our disease problems. The recognition of emerging conditions important to many and those fading in their significance must cross somewhere and thereby become difficult to evaluate. Those in the diagnostic laboratories in the swine raising areas would seem to have the best barometers for determining what the important disease problems of the period are and may become. However, when they are canvassed they are quick to point out their own misgivings about the information they either have or would seem to have when they voice an opinion. One develops the feeling that our most challenging problems are not necessarily designated by those diseases we seem to have the most. The most difficult problems seem to fall into patterns involving the properties of the infectious agent, the means of diagnosis, or the evaluation of the economic importance of the condition with and without secondary pathology. We recognize that all diseases are problems to someone either potentially or immediately.

The equation in disease problems is not complete without recognition of the role of nature. Environment, swine population, and factors which alter virulence of infectious agents must be considered in the summation of factors influencing the appearance or incidence of a given disease.

In the past year certain diseases, other than hog cholera which is so important to us that it occupies a significant segment of this program, have seemed to be of considerable concern to producers, practicing veterinarians and researchers. Our observations and experiences lead us to believe that the disease entities that are of greatest concern to the swine industry essentially fall into four categories. Enteritides, respiratory infections, and reproductive diseases plus erysipelas which with hog cholera represent the most severe of systemic infections.

**Enteritides**

There is little doubt that a group of diseases characterized primarily by the production of diarrhea in newborn and pre-weaning pigs remain as one of the more serious health problems facing the swine industry today.

As with the respiratory diseases, diarrheal conditions become increasingly important as the size of units increase. This is particularly true in large farrowing houses in which pigs are farrowed either continuously or in rapidly succeeding groups throughout the year. It is safe to say that every swine raiser using this type of system experiences losses due to diarrhea at times and in many herds it has become a continuing problem affecting practically every farrowing to a greater or lesser degree.

Our ability to identify these diseases of which we are reasonably sure there are several, leaves much to be desired. The virus causing transmissible gastroenteritis (4) is the only agent which has been well defined among those enteritides of great importance in young swine.
Ironically enough, in spite of the fact that more is known about the etiology, course, and pathology of TGE than of other neonatal diarrheas of pigs, we still do not have effective means of control or treatment of the disease. It has been demonstrated that immunity at least of a temporary nature may be transmitted to pigs by sows infected naturally or intentionally during pregnancy. It has also been shown that serum from swine that have recovered from the disease will neutralize the virus in vitro. But injections of immune serum or gamma globulin have not, in our hands, produced any resistance to the disease (5).

We know very little about how or where the virus is maintained between outbreaks. In our experience, TGE is exceedingly rare during the summer and autumn and only becomes a serious problem in late winter and spring.

One of the most serious limitations of research with TGE is that it has resisted all attempts to adapt it to tissue culture or to experimental animals other than swine. It is likely that progress with the disease will be slow until this is accomplished. Adaptation to tissue culture besides permitting characterization of the virus could lead to modification of the virus which could be used as an immunizing agent.

There remains an overwhelming majority of outbreaks of neonatal diarrhea in pigs which cannot be identified. McBryde (8) in 1934 wrote what appears to be the earliest description of the possible role of *Escherichia coli* in diarrhea of young pigs. It occurred as a diarrheal disease of varying mortality and morbidity which affected several large breeding herds in Los Angeles County, California. He associated the disease with *Escherichia coli* infection. Several writers (10, 13) since then have suspected that *E. coli* was concerned with gastroenteritis of young pigs but the ubiquitous occurrence of the organisms in the intestinal tract of normal pigs raised serious question as to its primary importance. Since development of serological typing of *E. coli* by Kauffmann (6) in 1944, important epidemiological information has been acquired linking certain strains to diarrheas of human infants and calves. Currently this technique is being applied in several laboratories and work has been published by the laboratories at Weybridge and Cambridge in England which leaves little doubt that certain strains of *E. coli* are associated with and probably of primary importance in diarrhea of young pigs.

**Respiratory Diseases**

Respiratory diseases continue to be of concern to swine raisers. Virus pig pneumonia (VPP) and atrophic rhinitis are two of the most important recognized diseases which provide challenge to the industry today.

The problems of VPP are similar to those of atrophic rhinitis. Although an agent for VPP has been described in a limited way, it seems certain that this and the swine influenza virus do not cause all of the pneumonias in swine. There is no rapid certain method of diagnosis. Influenza can be excluded by virological and bacteriological tests and VPP can be demonstrated by pig inoculation in that histopathology is reported to be distinctive. There are no serological tests for detecting carriers and no certain methods
for detecting carriers by physical examination. Therefore, the problem of control becomes one of restocking with swine free of the disease. This has been accomplished by raising litters with their sows in separate pens by Betts (2). It may also be accomplished apparently effectively by the “specific pathogen free” SPF process.

The main problems associated with Atrophic rhinitis are: that although a number of agents, Pasteurella multocida, Pseudomonas aeruginosa (12), Alcaligenes sp. (fecoal) (3) and chemical irritants (14) have been shown to cause atrophy—and a number of others—Mycoplasma hyorhinis, other PPLOs, Trichomonas sp. Hemophilus suis, Corynebacteria sp. Brucella bronchiseptica, and Spherophoruss necrophorus, have been associated with the disease (3), the etiology of the severe, growth depressing disease as is often encountered in the field has not been elucidated. What unknown agent, or combinations of agents or environmental factors are involved are not known. The lack of knowledge of etiology prohibits the development of rational diagnosis or therapy. Diagnosis presently is based only on the observation of lesions in the affected animal. While it is possible to observe these lesions in the severely affected animal by rhinoscopy or in less severely affected ones by radiograph, there is no means of detecting a possible carrier while it may not show turbinate atrophy. The only sure method of control is by repopulation with Atrophic rhinitis—free stock.

**Reproductive Disorders**

The reproductive capacity of swine is remarkable. When one considers the size of litters and the short gestation period, the potential number of new individuals is far greater than that realized annually.

Infectious diseases probably account for a large measure of the difference between the potential and that which is realized. The major manifestations of disorders are breeding difficulties, inapparent and frank abortions, and the birth of weak or dead pigs. While leptospirosis and brucellosis may be considered to be among the principal diseases resulting in reproductive disorders they surely are not the only causes. Much of our problem then is the development of knowledge concerning what other agents may be responsible. It is recognized that many febrile diseases may result in abortion and that there are hazards involved in the immunization of swine against hog cholera (11).

Leptospirosis presents a number of unknowns of interest to swine raisers, regulatory officials and research personnel.

There is a large number of animal species which are susceptible to a wide variety of serologically separable types of leptospiral organisms. This provides a remarkable pool of available reservoirs for the exposure of swine as they are susceptible to a number of serotypes of leptospiral organisms which can affect other species—Leptospira pomona, L. hyos, L. canicola, and L. icterohemorrhagica, for example.

Evaluation of serologically determined titers is difficult. The addition of clinical evidence of illness in the herd is required to help confirm the diagnosis. Feeder pigs carrying titers rarely show symptoms as 10 percent of
market hogs, on random sampling, have been shown to have significant titers. Titers, usually considered to be diagnostic may remain as long as 14 months (9). Some measure of titer may last for years. At present, when titer is found in feeder pigs, an owner can be informed that his animals have had experience with leptospirosis but that the significance is not known.

The identification of swine shedding organisms in the urine may be most difficult. Renal shedders may exist for six months or longer. The organisms will survive for about one hour in voided urine which creates the problem of laboratory identification. Treatment of these carrier animals at present is impractical because the antibiotics are not effective or the doses necessary are so high that they are toxic before they are effective.

Available immunizing agents will provide immunity for six to nine months and thereby are useful for protection of sows during gestation. Nonvaccinated sows, infected early in gestation, develop titers and farrow normal offspring, while those infected at mid-pregnancy or later develop titers and farrow few normal pigs, most of which are so devitalized that they are dead or survive less than 24 hours. From 10 to 100 percent morbidity may be found in sows which when coupled with symptoms and the farrowing of stillborn or weak pigs which die within 24 hours provides the basis for the recognition of the losses due to infection with leptospiral organisms. From the public health standpoint research workers wonder what part the disease in swine plays in the incidence of leptospirosis in man.

**Systemic Infection**

The organism causing swine erysipelas disease is widespread in nature appearing for the most part as an innocuous saprophyte (15). However, it may suddenly become virulent and cause great havoc as an agent producing an acute febrile, disease with high morbidity and mortality. In alkaline soils according to Aitken (1), the incidence of acute infections has been shown to be high, in other areas the chronic form is common. Following a flood in areas where chronic form is common the losses due to acute erysipelas may increase markedly.

While the use of live culture and antiserum or bacterins may be of value in warding off acute infections their use in the past few years has not seemed to reduce the amount of chronic polyarthritis in swine. Arthritis continues to be one of the foremost causes of condemnations of swine carcasses (16).

Chronic erysipelas is manifested by swollen sore joints but it is certainly not the only cause of polyarthritis. One of the problems in erysipelas is that erysipelas has been a "catchall" diagnosis for polyarthritis of swine which may have been due to corynebacteria, streptococci, staphylococci, PPLO, or brucella organisms (7). Studies of experimentally produced erysipelatous polyarthritis at Purdue University have indicated that if penicillin is to be of value it must be administered within two weeks of exposure to the organism.

The researcher finds this organism difficult to work with. Animals of uniform susceptibility are very difficult to obtain. If mice and pigeons, which are highly susceptible, are used the results although interesting, are difficult
to relate to the disease in swine. A second problem for the research group is
the fact that there are no consistently good serological methods for evaluating
immunity. When the strength of immunity is sought in swine following
experimental immunization it is found that endpoints are obscure because of
the variation of expression of the disease in swine.

Acute erysipelas resembles hog cholera as has long been recognized. In
recent years with the use of modified hog cholera virus the less severe forms
of erysipelas likewise are confused with the less severe and more chronic
forms of hog cholera. This situation has caused much unhappiness for
producers and veterinarians alike.

In the coming decade it is estimated that we will need to raise 30 percent
more livestock and that it will probably be done with about five percent less
land under cultivation. Trends toward specialization of livestock enterprise
will continue with fewer classes of livestock on each farm but in larger
numbers. Inefficient producers will retire with larger better managed enter-
prises becoming more numerous. Because of these economic factors requiring
increased concentration of a larger livestock population, enteric and respira-
tory diseases will remain at least as important to the swine industry as they are
today. More research must be conducted to separate out disease entities in
these broad disease problems and to provide means of prevention and control.
Reproductive disorders deserve greater attention as producers will seek
greater farrowing efficiency to meet the demands of competition in the market.

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   VII. Re-exposure of Pregnant Sows with Leptospira pomona. Am. J. Vet. Res. 21
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REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


Previous reports of this Committee are comprehensive in their reference to the relative incidence of the various diseases of swine, therefore, this Committee reaffirms those reports as any spontaneous decline in incidence is unlikely whereas some increase is most probable. It is further noted that the very important recommendations of the 1959 report have not attained fulfillment, therefore, this Committee reaffirms the report.

Although this report covers transmissible diseases of swine other than hog cholera, we believe that any recommendations herein are applicable and that the current interest in a National Hog Cholera Eradication Program offers an excellent opportunity for the development of a “Uniform Program” for the control and eradication of other diseases of swine. More specifically, the disease of virus pneumonia, atrophic rhinitis, the enteritis complex both of baby pigs and older animals and the acute respiratory infections deserve first consideration.

We are of the opinion that any program, to be effective, must be uniform and cooperative; that it must have the interest and support of all segments of the industry and that the decreasing margins of profit in swine production should stimulate that interest and support.

Breeders of swine, whether they be registered or commercial, can no longer avoid their individual responsibility, and therefore, must discontinue the sale of animals for breeding purposes when transmissible diseases are known to exist in their herds. The decision must soon be made to adopt sound management and sanitation practices within individual operations for the control and eradication of disease, or, if necessary, to avail themselves of Pathogen-Free breeding stock for a start anew.

We believe that the uncontrolled movement of feeder pigs has contributed greatly to the rapid spread of swine diseases and for the introduction of disease into breeding herds that were not protected by effective management and sanitation practices. Especially is this true in the combination breeding and feeding operation.

Swine breeders, feeders, dealers, market operators and regulatory officials can no longer ignore the marketing process which extends the movement of swine through a series of markets and dealers premises and requiring from a few days to several weeks for such swine to move from producer to feeder or
breeder. It is time that all concerned recognize and accept the fact that
the modern day pig is being subjected to excessive and unnecessary stress
and exposure to disease and that both must be reduced to a minimum in an
improved marketing process that is more direct and less time consuming.

In the interest of developing effective programs for the control of swine
diseases we offer the following basic recommendations:

1. That a Swine Disease Section be established with the Animal Disease
   Eradication Division of the Agriculture Research Service and that
   adequate funds be provided for its effective contribution to cooperative
   swine disease control and eradication.

2. That the Animal Disease Eradication Division immediately adopt regu-
   lations for the interstate movement of swine.

3. That uniform state laws and regulations be adopted for better control
   over swine movements into and within the various states; for the
   licensing of auction markets, dealers and truckers.

4. That adequate diagnostic facilities be provided in each state.

5. That each state enact laws or regulations restricting the sale of virulent
   hog cholera virus.

6. That there be a considerable increase in funds allocated to the Agri-
   culture Research Service for more intensive research in diseases of
   swine; and for the development of diagnostic tests and effective vaccines
   where indicated.

7. That the states give attention to vehicle sanitation and that laws be
   adopted to implement such a program.

8. That the State Extension Services develop educational programs in
   swine management and sanitation in cooperation with their respective
   state veterinary associations, schools of veterinary medicine and
   regulatory officials.

9. That the United States Livestock Sanitary Association authorize the
   Committees on Transmissible Diseases of Swine and Nationwide Eradi-
   cation of Hog Cholera to develop “Uniform Methods and Rules” for
   the control and eradication of swine diseases and that these be presented
   to the Association at its next meeting.

10. That research on hog cholera be intensified.

This Committee endorses the Report of the Committee on Nationwide
Eradication of Hog Cholera.
PROGRESS REPORT OF THE EXPERIMENT ON THE ERADICATION OF
HOG CHOLERA IN THE FLORIDA PILOT TEST AREA—
FISCAL YEAR 1960

M. R. ZINOBER, D.V.M. AND SEIBERT L. BERLIN, V.M.D.*

Live Oak, Florida

The reports to this Association in 1958† and 1959‡ described the establishment of Suwannee County, Florida, as a pilot test area for the eradication of hog cholera by the Florida Livestock Board and the construction of the Florida Hog Cholera Research Station by the Animal Disease and Parasite Research Division of the United States Department of Agriculture. In these reports, the objectives of the program, methods of operation of the Station, and results of our investigations were also given.

In the reports mentioned above, it was stated that “. . . the Hog Cholera Research Station continued investigations in two general areas to determine (1) the immunogenic efficiency of all types of modified live virus vaccines administered with a minimum dose of 15 ml. of anti-hog cholera serum, and (2) the status of eradication of the disease from the pilot test area by attempting to establish the incidence of hog cholera in the area.” These objectives are still valid.

The Immunogenic Efficiency of Modified Live Virus Vaccine.—During fiscal year 1960, 31,099 swine in 992 herds were vaccinated. Table 1 gives the breakdown of these data according to the type of vaccine used. Since there are approximately 60,000 swine in Suwannee County, these figures represent vaccination of 51.8 percent of the swine in the county. This represents a slight drop in coverage from the figure for fiscal year 1959, which was 54.9 percent.

Also in fiscal year 1960, 658 vaccinated hogs representing 340 herds were challenged. The hogs were challenged with one ml. of Station virus s.n. one as described in our report to this Association in 1958. The latest titration of this virus, which was completed on March 18, 1960, showed that it had a minimum lethal dose of \(10^{-5}\) ml. Table 2 gives the results of these challenges according to the type of vaccine used. For comparison, all the data collected from the commencement of the work in April 1957, is also shown in

* Florida Hog Cholera Research Station, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Live Oak, Florida.


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ERADICATION OF HOG CHOLERA IN FLORIDA

TABLE 1
Vaccination in Suwannee County
Fiscal Year 1960

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Herds</th>
<th>Hogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage of Total</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>362</td>
<td>36.5</td>
</tr>
<tr>
<td>Porcine Origin</td>
<td>396</td>
<td>39.9</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>234</td>
<td>23.6</td>
</tr>
</tbody>
</table>

| Total          | 992    | 100.0 | 31099   | 99.9  |

The table. This includes the data for the last three months of fiscal year 1957 and all of fiscal years 1958 and 1959 as well as all of fiscal year 1960. The most salient feature of the table is the steady decline in percentage of adequately protected hogs vaccinated with all three types of vaccine from fiscal year 1957 to fiscal year 1959. In fiscal year 1960, lapine origin and tissue culture vaccines tended to recover their levels of protective potency, although they did not quite reach their levels of fiscal year 1957. The level of protective potency of porcine origin vaccine, however, continued to decline in fiscal year 1960. (The slight increase in percentage of adequately protected hogs vaccinated with porcine origin vaccine in fiscal year 1958 over fiscal year 1957 is without statistical significance.)

In order to present a more detailed picture of the immunogenic efficiency of the vaccines, data were compiled on the basis of 12-month periods. The data presented in Table 3 are the total for the periods indicated, each period representing 12 months. Thus, the first line in Table 3 shows that for the entire 12-month period from April 1, 1957, to March 31, 1958, inclusive, a total of 417 hogs which had been vaccinated with lapine origin vaccine were challenged and 390 of them, or 93.5 percent, were adequately protected. The data in the columns headed "Porcine Origin" and "Tissue Culture" give the results for the same time period for hogs vaccinated with these types of vaccine. In the same way, the second line in the table gives the total challenge results for the entire 12-month period from May 1, 1957, to April 30, 1958, inclusive. In order to present these data more effectively, the percentages only have been plotted on Graph 1.
TABLE 2

Results of Virus-Challenge of Pigs Vaccinated With Modified Live Virus Vaccine

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Fiscal Year 1957*</th>
<th>Fiscal Year 1958</th>
<th>Fiscal Year 1959</th>
<th>Fiscal Year 1960</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number†</td>
<td>Percent</td>
<td>Number†</td>
<td>Percent</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>65/67</td>
<td>97.0</td>
<td>389/415</td>
<td>93.7</td>
</tr>
<tr>
<td>Porcine Origin</td>
<td>79/83</td>
<td>95.2</td>
<td>377/388</td>
<td>97.2</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>46/47</td>
<td>97.9</td>
<td>422/450</td>
<td>93.8</td>
</tr>
<tr>
<td>Total</td>
<td>190/197</td>
<td>96.4</td>
<td>1188/1253</td>
<td>94.3</td>
</tr>
</tbody>
</table>

* April, May and June, 1957, only.
† Numerator—number of hogs adequately protected.
Denominator—number of hogs challenged.
### Table 3

**Challenge of Vaccinated Hogs in Suwannee County by Twelve-Month Periods**

<table>
<thead>
<tr>
<th>Time Period (All Dates Inclusive)</th>
<th>Lapine Origin</th>
<th>Porcine Origin</th>
<th>Tissue Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number* of Hogs</td>
<td>Percentage Adequately Protected Hogs</td>
<td>Number* of Hogs</td>
</tr>
<tr>
<td>4/1/57—3/31/58</td>
<td>390/417</td>
<td>93.5</td>
<td>419/434</td>
</tr>
<tr>
<td>5/1/57—4/30/58</td>
<td>403/429</td>
<td>93.9</td>
<td>417/431</td>
</tr>
<tr>
<td>6/1/57—5/31/58</td>
<td>381/407</td>
<td>93.6</td>
<td>377/388</td>
</tr>
<tr>
<td>7/1/57—6/30/58</td>
<td>389/415</td>
<td>93.7</td>
<td>377/388</td>
</tr>
<tr>
<td>8/1/57—7/31/58</td>
<td>404/431</td>
<td>93.7</td>
<td>380/393</td>
</tr>
<tr>
<td>9/1/57—8/31/58</td>
<td>370/401</td>
<td>92.3</td>
<td>356/369</td>
</tr>
<tr>
<td>10/1/57—9/30/58</td>
<td>347/377</td>
<td>92.0</td>
<td>371/387</td>
</tr>
<tr>
<td>11/1/57—10/31/58</td>
<td>320/349</td>
<td>91.7</td>
<td>316/335</td>
</tr>
<tr>
<td>12/1/57—11/30/58</td>
<td>292/314</td>
<td>93.0</td>
<td>306/328</td>
</tr>
<tr>
<td>1/1/58—12/31/58</td>
<td>279/298</td>
<td>93.6</td>
<td>270/293</td>
</tr>
<tr>
<td>2/1/58—1/31/59</td>
<td>244/264</td>
<td>92.4</td>
<td>248/273</td>
</tr>
<tr>
<td>3/1/58—2/28/59</td>
<td>219/246</td>
<td>89.0</td>
<td>212/238</td>
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<td>204/233</td>
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<td>204/233</td>
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<td>192/223</td>
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<td>83.4</td>
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<td>81.5</td>
<td>176/215</td>
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<td>80.8</td>
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<td>85.1</td>
<td>132/169</td>
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<td>2/1/59—1/31/60</td>
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<td>90.9</td>
<td>123/182</td>
</tr>
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<tr>
<td>7/1/59—6/30/60</td>
<td>311/358</td>
<td>86.9</td>
<td>146/237</td>
</tr>
</tbody>
</table>

* Numerator—number of hogs adequately protected; denominator—number of hogs challenged.

† Eleven months only. No data for July 1959.
Graph 1 demonstrates clearly the dramatic and continuous decline of the percentage of pigs adequately protected by all the vaccine types after virus-challenge and the continued decline of porcine origin vaccine while lapine origin and tissue culture vaccines generally tended toward recovery of their level of protective potency.

In order to find the cause of the decline of immunogenic efficiency a study was undertaken this year of the time the vaccine was used in relation to its proximity to the expiration date of the particular serial number being used. In a preliminary study of this question, it seemed that there was an inverse variation between the proximity of some serials of vaccine to their expiration dates and their immunogenic efficiency as shown in Table 4. This preliminary study revealed that the percentages of adequately protected pigs were markedly lower when certain serials of vaccine were used within six months of their expiration dates compared to their use more than six months prior to their expiration dates. It is especially noteworthy that this loss of immunogenic efficiency within the last six months of their expiration dates was demonstrated to a significant degree by all three types of vaccines.
<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Number of Serials</th>
<th>24-7 Months Inclusive</th>
<th>6-0 Months Inclusive</th>
<th>Decrease</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number* of Hogs</td>
<td>Percentage of Adequately Protected Hogs</td>
<td>Number* of Hogs</td>
<td>Percentage of Adequately Protected Hogs</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>21</td>
<td>652/732</td>
<td>89.1</td>
<td>126/154</td>
</tr>
<tr>
<td>Porcine Origin</td>
<td>17</td>
<td>656/765</td>
<td>85.8</td>
<td>63/113</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>22</td>
<td>237/256</td>
<td>92.6</td>
<td>520/625</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>1545/1753</td>
<td>88.1</td>
<td>709/892</td>
</tr>
</tbody>
</table>

* Numerator—number of hogs adequately protected. Denominator—number of hogs challenged.
Eradication of Hog Cholera.—The status of eradication of hog cholera from the pilot test area has been of the greatest concern during the past fiscal year. In 1956, 13 hog cholera suspect cases were reported. In 1957, there were 18 such cases. During the first six months of 1958, 14 hog cholera suspect cases were reported. In fiscal year 1959, 38 cases were reported, 16 of which were confirmed by animal inoculation tests. In fiscal year 1960, 46 cases were reported, 24 of which were confirmed. It is estimated from these data that there has been about a 50 percent annual increase in the incidence of hog cholera for the pilot test area during the past five years. Herds suspected of having hog cholera by the practicing veterinarian were quarantined immediately by an officer of the Florida Livestock Board after he was supplied the necessary information. After the quarantine was applied, visits to the farm were made periodically by Station veterinary personnel to elicit certain vital information, the most important of which was the source of infection. Information was sought in the following general areas: (1) history of the herd before the current outbreak, (2) observations made on the farm at the time of the first investigations, and (3) observations and history of the herd from the time of the issuance of the quarantine to the time of its release.

At the time of the first suspicion of hog cholera in a herd, the practicing veterinarian collected tissue samples or a suitable morbid pig, which he submitted to the Station for test. The tests were conducted in the manner described in our report to this Association in 1959. The results of these tests are given in Table 5.

### Table 5

**Confirmed Cases of Hog Cholera in Suwannee County in Fiscal years 1959 and 1960**

<table>
<thead>
<tr>
<th></th>
<th>Fiscal Year 1959</th>
<th></th>
<th>Fiscal Year 1960</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated</td>
<td>Non-Vaccinated</td>
<td>Vaccinated</td>
<td>Non-Vaccinated</td>
<td>Number</td>
</tr>
<tr>
<td>Farm-raised</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Purchased</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>4</td>
<td>10</td>
<td>14</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 5 shows a marked increase in the number of confirmed positive cases of hog cholera from 16 in fiscal year 1959 to 24 in fiscal year 1960, an increase of 50 percent. The decrease in vaccine efficiency, the decrease in vaccination coverage in the pilot test area noted above, from 54.9 percent in fiscal year 1959 to 51.8 percent in fiscal year 1960, and the increased incidence of the disease in neighboring counties probably all contributed to the increased incidence in Suwannee County. However, it is believed the movement of swine through public markets has been the major factor contributing to the disease incidence increase. Efforts by means of radio,
ERADICATION OF HOG CHOLERA IN FLORIDA

newspapers, and mail circulation through the office of the county agricultural agent had a favorable effect on the vaccination program. Table 5 shows also that of the 40 confirmed cases, 21 (52.5 percent) occurred in farm-raised herds and 19 cases (47.5 percent) were associated with purchases.

A study of the 22 confirmed cases in vaccinated swine in fiscal years 1959 and 1960 revealed the following: In 12 cases the outbreaks were associated with the introduction of purchased swine. Of the 10 confirmed cases in vaccinated, farm-raised swine, in two cases the pigs were sick at the time of vaccination; in two cases the outbreaks occurred within one week after vaccination, and in six cases the outbreaks occurred from 18 to 88 days following vaccination.

An important phase of the eradication problem in Suwannee County has been the need to trace swine that are moved from the public market to the farm. Market reports have been submitted by inspectors of the Florida Livestock Board to the Florida Hog Cholera Research Station weekly from all public markets in Suwannee County and from most markets in neighboring counties where Suwannee County farmers are likely to market hogs. The data from these markets include the names and addresses of the sellers of the hogs, the numbers of vaccinated and nonvaccinated slaughter hogs and the numbers of vaccinated and nonvaccinated feeder pigs.

In addition to the market reports mentioned above, the local public market has been furnishing us with the names and addresses of the buyers of pigs which were not intended for immediate slaughter. By means of these two sets of information together with the vaccination records furnished to the Station routinely by the local practicing veterinarians, it has been possible to trace the movement of vaccinated and nonvaccinated swine from the public market back to the farm. Although the Florida Livestock Board has had a regulation in effect since July 1, 1958, that all swine passing through a public market and not intended for immediate slaughter must be vaccinated before being removed from the market premises, there have still been swine raisers who continue to violate this regulation. By the use of the information mentioned above, it would be possible to identify these violators, locate them, and require them to abide by the regulation calling for vaccination of their purchased hogs.

Throughout the year, the locations of farms in Suwannee County on which hog cholera was confirmed were plotted on a large aerial photograph of the county. The locations of these farms showed that the outbreaks were usually geographically localized and that the center of localized infection shifted from time to time during the year. This suggests interfarm transmission of the infection within each localized area.

Although the increasing incidence of hog cholera and the decreasing vaccination coverage in Suwannee County are discouraging factors, information is being developed which, it is hoped, will be useful for the eventual eradication of this wholly unnecessary disease in the pilot eradication test area, and, by extension, throughout the nation.
STUDIES ON MODIFIED VIRUS VACCINES FOR HOG CHOLERA

II. REACTIVATION BY SERIAL PASSAGE

J. P. Torrey, D.V.M., M.S., B.S.*; M. R. Zinober, D.V.M.†; W. C. Amtower, B.S.*

Since the introduction of modified live hog cholera virus vaccine in 1951, there has been a gradual decrease in the sale of virulent virus (1) and no major outbreaks of hog cholera throughout the swine population. The use and acceptance of these vaccines have made it practical to prohibit the sale of virulent virus in more than 30 states; to some extent, the vaccines have made it possible to start an eradication program. Like all other biologics, however, modified live virus vaccines are not perfect and will not give 100 percent protection under all field conditions. As soon as these vaccines became widely used, so-called “breaks” or death losses in some vaccinated farm herds were reported. It is very difficult to account for these losses since the viruses from which the vaccines were made were attenuated so that susceptible test pigs did not show signs of the disease after vaccination.

These losses following vaccination with modified live virus vaccines create three major questions:

1. Would the modified vaccine viruses pass from injected animals to susceptible contacts?
2. Would the viruses continue to immunize the pigs without regaining virulence when serial passages were made through pigs?
3. Would the characteristics of the viruses be changed by passage through pigs?

This paper reports experiments conducted to give some information on these questions. The experiments were carried out over a four-year period.

MATERIAL

Five commercial vaccines from three different sources were purchased and used in these tests. Four vaccines were of rabbit origin and one was of porcine origin. A sixth virus was isolated from farm pigs which sickened after vaccination with a vaccine of rabbit origin.

The susceptible pigs used for passage of the viruses weighed from 40 to 70 pounds, and were purchased directly from farms located in an area where the incidence of hog cholera was very low, and vaccination against hog cholera was rarely practiced. The immunity of representative pigs from each

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† Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Live Oak, Florida.

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accession lot was challenged with two cc. of regular virulent hog cholera virus. The virus used for challenge was the laboratory strain, which had been kept for a number of years, and had been tested repeatedly and found free of “variant characteristics” (2). One serial of challenge virus was used throughout each series of passages of vaccine.

The pigs were kept isolated in individual houses or in isolation pens constructed so that intake air was filtered, and all waste material was decontaminated.

METHODS

The commercial modified live virus vaccines were reconstituted and injected as prescribed by the manufacturer, except that no anti-hog cholera serum was given with the vaccine. Daily observations of each pig were made and recorded. Five to seven days after the injection of vaccine, a blood sample was drawn from the anterior vena cava, defibrinated by shaking, and five cc. injected intramuscularly into another susceptible pig. After five days the second pig was bled, and defibrinated blood injected into a third pig in the same manner. This process was repeated each five to seven days interval until the desired number of passages were made. The immunity of each pig which had no reaction to the injection and remained normal was challenged with two cc. of virulent hog cholera virus 21 days after the initial injection (except where noted). They were held 14 days longer to determine their immunity. Those pigs that had no reaction were released as “immune.” Those pigs that became sick after challenge were held until they recovered or died.

Post-mortem examinations were made of all pigs that died. Bacteriological examinations were made of the heart, lungs, liver, spleen, and kidneys; any bacteria isolated were identified. Those pigs that had a reaction after the initial injection of blood were held until they died or recovered. Seven days after they had returned to normal, their immunity was challenged with two cc. of virulent hog cholera virus; if no reaction occurred, they were released in 14 days as immune.

Some of the tests were made by using three pigs for each blood sample. One pig was placed in the pen as a contact control, and the other two were injected with the pooled defibrinated blood of the two previously injected pigs. The contact control pig determined if the virus was being eliminated from the injected pigs.

RESULTS

A tabulation of the following results is shown in Table 1.

I. Vaccine of Rabbit Origin

The first serial passages of lapinized vaccine (1A) were made three years after this type of vaccine was placed on the market for use by the veterinarian. Two pigs were used for each passage, and a control was placed in each pen. There was no reaction in any of the pigs injected with the first, second and
third passage of blood. The injected pigs and the susceptible controls were all immune when challenged with virulent hog cholera virus. This showed that the virus did pass from the vaccinated animals to the contact animals. There were no reactions in the fourth and fifth passages. However, only one of the pigs was immunized by the fourth passage. One injected pig and the control died after challenge. Both of these pigs had typical lesions of hog cholera and *Salmonella choleraesuis* was isolated from the organs. The injected pigs as well as the control pig were immune in the fifth passage without having any reactions. In the sixth passage, one injected pig had no reaction and was immune when challenged. The other injected pig sickened on the seventh day after injection and died on the thirteenth day. Typical lesions of hog cholera were observed at post-mortem and bacteriological examination was negative. The control pig sickened on the thirteenth day and died on the sixteenth day. Apparently the control was infected from the injected pig. In the seventh passage, both pigs sickened on the seventh day and died on the ninth day after injection. In the eighth passage, both pigs sickened on the fifth day and died on the seventh and ninth days after injection. A second eighth passage was made on a different lot of pigs. One of the injected pigs sickened on the seventh day after injection and died on the eighth day. It had typical lesions of hog cholera and the bacteriological examinations were negative. The other injected pig remained normal and was immune when challenged with virulent virus. The control pig in this passage died when challenged on the twenty-first day. A ninth passage was made and both pigs sickened on the fourth day and died on the sixteenth and twenty-second days respectively. Both pigs had lesions of hog cholera and no bacteria were isolated.

Virus from the eighth passage was tested for variant characteristics (2) by the injection of two cc. intramuscularly simultaneously with 15 cc. of serum into each pig. These pigs had a reaction which lasted six days. They recovered and proved to be immune by challenge with two cc. of virulent virus 46 days after treatment. This test indicates that some change had taken place in the virus other than an increase in its virulence.

A second serial passage (1B) was made of this same brand of vaccine two and a half years later with a currently produced serial lot of vaccine. The size of the available isolation pens made it necessary to use only one pig for each passage instead of two pigs and a control. Thirty-five serial passages were made of this vaccine. All pigs were bled on the fifth day after injection, and blood was injected into another susceptible pig. The immunity of all pigs was challenged 21 days after injection. There were no reactions in the first 17 passages and all the pigs were immune to challenge. The pig in the eighteenth passage sickened on the fifth post-vaccination day with a temperature of 106.8 F. and died on the seventeenth day. Typical lesions of hog cholera were observed on post-mortem examination and bacteriological tests were negative. In the nineteenth passage, the pig was sick on the tenth day and on the eleventh day had a temperature of 104.8 F. The pig returned to normal on the twelfth day and proved to be immune when challenged with virus. The pigs in passages 20, 21, 22, and 23 were normal after
injection and resisted immunity challenge. The pig in the twenty-fourth passage was sick the thirteenth, fourteenth, fifteenth, and sixteenth days after injection. It recovered and was immune when challenged. The pig in the twenty-fifth passage died from an accident. In the twenty-sixth passage, the pig sickened on the seventh day and died on the eleventh day after injection. Typical lesions of hog cholera were present; bacteriological tests were negative. In the twenty-seventh passage the pig remained normal and was immune. In the twenty-eighth and twenty-ninth passages, the pigs were slightly sick for two days and were immune when challenged. Pigs in passages 30 to 34 inclusive sickened on the sixth day after injection, and were sick for three to six days. Temperatures ranged from 104.0 to 105.0°F. All of these pigs returned to normal and were immune. The pig in the thirty-fifth passage sickened on the sixth day after injection with a temperature of 107.0°F. and died on the fifteenth day. Typical lesions of hog cholera were observed at post-mortem examination, and *Salmonella choleraesuis* was isolated from the organs.

II. Vaccine of Rabbit Origin—Different Producer

A second vaccine of rabbit origin (II) was serially passaged 27 times. Each passage was made in one pig, and there were no contact controls. No reactions were observed in the first nine passages and all pigs were immune to challenge. In the tenth passage, the pig sickened on the tenth day with a temperature of 105.8°F. and died on the thirteenth day. Typical lesions of hog cholera were present and *Salmonella choleraesuis* was isolated from the organs. In the eleventh passage, the pig had a temperature of 106.0°F. on the fifth day and remained sick until the nineteenth day. It recovered and was immune. In the twelfth passage the pig was sick the fifth and sixth days with a temperature of 104.0°F. This pig was immune to hog cholera. In the thirteenth passage, the pig was sick on the fifth post-injection day with a temperature of 104.8°F. It had recovered by the eighth day and was immune when challenged. In the fourteenth passage, the pig was sick from the seventh to the sixteenth day. It recovered and was also immune. The fifteenth passage pig was sick the ninth and tenth days. It was immune when challenged. The sixteenth passage pig died on the eighth day after injection and was not visibly sick. The lesions observed were not typical of hog cholera, and bacteriological tests were negative. The seventeenth passage pig was sick 18 days. It recovered and was immune when challenged. The eighteenth passage pig was sick on the fourth day after injection and died on the tenth day. Typical lesions of hog cholera were present. Streptococci were isolated from the lungs. The nineteenth passage pig was sick one day and died on the eighth day after injection. Typical lesions of hog cholera were observed; bacteriological tests were negative. The pig on the twentieth passage had a severe reaction from the seventh to the tenth days after injection and was immune. The pigs on the twenty-first, and twenty-second passages had slight reactions on the seventh to ninth days and were immune when challenged. The twenty-third passage pig was sick the sixth day and died on the ninth
day. Typical lesions of hog cholera were observed and bacteriological tests were negative. The twenty-fourth passage pig was sick on the third day after injection and died the thirteenth day. Typical lesions of hog cholera were observed. The twenty-fifth passage pig was sick two days and died from the bleeding operation. The twenty-sixth passage pig was sick on the seventh day with a temperature of 106.6 F. It died on the twelfth day. Lesions typical of hog cholera were observed and bacteriological tests were negative. The twenty-seventh passage pig was sick on the sixth day, and died on the eleventh day. Typical lesions of hog cholera were observed.

III. Vaccine of Rabbit Origin

A third vaccine from another producer (III) was tested by 45 serial passages. The first 18 passages produced no reactions in the pigs and all the pigs were immune when challenged. No contact control pigs were used in this series of passages. In the nineteenth passage the pig had a reaction only the eighth day with a temperature of 104.8 F. In the twentieth passage the pig had a reaction only the fifth day with a temperature of 104.3 F. In the twenty-first and twenty-second passages the pigs were normal. In the twenty-third passage the pig was sick two days with a temperature of 104.0 F. In the next four passages, 24 to 27 inclusive, the pigs had no reactions. On the twenty-eighth passage the pig was sick two days with a temperature of 104.5 F. In the twenty-ninth passage the pig was sick five days with a temperature of 106.0 F. In the thirtieth passage the pig was sick five days with a temperature of 105.8 F. In the thirty-first passage the pig was sick eight days with a temperature of 106.5 F. In the next three passages, 32, 33, and 34, the pigs had no reaction. In the thirty-fifth passage the pig was sick 16 days. In passage 36 the pig was sick 20 days. In passages 37, 38, and 39 the pigs had slight reactions one day. In passage 40 the pig was sick four days. In passage 41 the pig had no reaction. In passage 42 the pig was sick nine days. In passage 43 the pig was sick on the seventh to the tenth days. In passages 44 and 45 the pigs had no reactions. All of the pigs used in this series of 45 passages were normal at the time of challenge and all proved to be immune.

IV. Vaccine of Swine Origin

The passages with this vaccine were made in 1954. Two pigs injected with the vaccine had reactions beginning on the fourteenth day and lasting eight days. They were normal after the twenty-first day, and were immune when challenged. Pigs on the second passage sickened on the seventh day after injection and had recovered by the fourteenth day. They were immune when challenged. The controls of the first two passages remained normal and were immune when challenged. The two pigs on the third passage sickened on the seventh day after injection. One pig had recovered by the twenty-fifth day and was immune. The other pig died on the sixteenth day. Typical lesions of hog cholera were present and Salmonella choleraesuis was isolated. The control pig had a slight reaction on the twentieth day but recovered and was immune. There was no reaction in the pigs on the fourth passage, and both the injected
pigs and the control were immune when challenged. In the fifth passage both injected pig sickened on the eighth day. One pig died the fifteenth day, typical lesions of hog cholera were observed and *Salmonella choleraesuis* was isolated. The other pig had recovered by the twenty-eighth day and was immune. The control pig sickened on the eleventh day and died the twenty-first day after the other pigs were injected. Typical lesions of hog cholera were observed. In the sixth passage one pig sickened on the fifteenth day and died on the nineteenth day. The other pig remained normal and was immune when challenged. The control pig sickened on the seventeenth day and died on the nineteenth day. In the seventh passage both pigs sickened on the ninth day. One pig died the eleventh day and the other pig died the twenty-sixth day. The control sickened on the sixteenth day and died on the nineteenth day after the other two pigs were injected. In the eighth passage both pigs sickened on the fourth day. One died the eighth day, and the other died the twelfth day. The control pig sickened the eleventh day and died the twenty-third day after the other pigs were injected. Typical lesions of hog cholera were observed in all the pigs that died from the sixth to the eighteenth passages.

No indication of variant characteristics (2) could be demonstrated in this virus. In every passage the virus was transferred from the injected pigs to the controls by contact.

V. Field Strain of Virus

The relationship between the death losses in farm herds after vaccination with modified vaccines and the virus used in the vaccine has been hard to establish. Viruses isolated from these herds may immunize experimental pigs without causing any reactions. A virus was obtained from a herd of 131 head of 30-40 lb. pigs, raised and vaccinated by the owner. Three sick pigs were observed six days after vaccination. All of the pigs were sick in 17 days. A diagnosis of hog cholera was made at a state diagnostic laboratory. Peripheral hemorrhages of the lymph nodes, petechial hemorrhages in the kidney and urinary bladder and infarcts in the spleen were the lesions observed. The practicing veterinarian treated the pigs with penicillin without beneficial results. Anti-hog cholera serum was given and the pigs recovered in a few days.

A filtered saline suspension of macerated tissue from one of these pigs was injected into four susceptible pigs. The four pigs remained normal for 14 days at which time they were challenged. All four pigs remained normal 14 days after challenge and were released as immune. These pigs were bled eight days after injection with the tissue filtrate and two cc. of pooled defibrinated blood were injected into each of two susceptible pigs. The pigs in the first, second, third and fourth passage had no reaction after injection. All of the injected pigs and the controls in these four passages were immune when challenged on the twenty-first day. In the fifth passage the injected pigs were sick on the eighteenth day but had recovered by the twenty-fifth day and were immune. The control pig had a severe reaction and was immune.
In the sixth passage there was no reaction in either pig, and both pigs were immune. The control pig had no reaction and was not immune. In the seventh passage one pig was sick on the sixth day and died on the eighteenth day. The other pig remained normal and was immune. The control had no reaction and was immune. In the eighth passage both pigs were sick on the seventh day and had recovered by the twelfth day. Both pigs were immune. The control pig had no reaction and was immune. In the ninth passage one pig had a reaction for ten days and recovered. The other injected pig and the control remained normal and all three pigs were immune. In the tenth passage one pig had a reaction for six days and recovered. The other injected pig and the control remained normal and all three pigs were immune. In the eleventh passage none of the pigs had a reaction. Both injected pigs were immune. The control pig had no reaction and was not immune. In the twelfth passage one injected pig sickened on the sixth day and died the thirteenth day. The other injected pig had no reaction and was immune. The control pig was sick on the eleventh day after the other pigs were injected and died the seventeenth day. In the thirteenth passage one pig was sick on the tenth day and died the thirteenth day. Typical lesions of hog cholera were observed. The other injected pig had no reaction and was immune. The control pig had no reaction and was not immune. In the fourteenth passage both pigs were sick on the eighth day, one died in three days, and the other recovered in four days and was immune. The control pig sickened on the tenth day. It was sick 14 days and died.

DISCUSSION

When these types of experiments are conducted they necessarily cover a considerable length of time, and involve many variable factors which must be considered in the results. The inherent variations in different lots of pigs has some effect on the results. Latent infections in the pigs, weather conditions and methods of handling the pigs must be considered.

The rabbit origin type of vaccines had the same general pattern of response to serial passage in all four samples tested. None of these vaccines produced reactions in the pigs in the first passages. One vaccine required five passages, one required 17 passages, one required nine passages and another required 18 passages before reactions were visible in the pigs. All of the rabbit origin vaccines regained virulence when they were passed a sufficient number of times. The first reactions in most cases were more mild, of shorter duration and the pigs would recover more quickly. As the number of passages increased the severity of the reactions increased and death occurred in three of the four samples tested. The other sample did not cause any pigs to die. Some vaccines became pathogenic somewhat gradually, while others increased in pathogenicity quickly. There seemed to be an alternate pathogenic and nonpathogenic phase in these vaccines. In one, two, or three serial passages the pigs would have a reaction. In some cases the pigs would die. Then in the next two to four passages the pigs would have no reactions but would be immune when challenged.
In all but four passages, where pigs were used for controls or contact pigs, they were either immune or died before being challenged. It is therefore evident that under these experimental conditions the viruses did pass from the injected pigs to the contact controls. Those four controls which were not immunized apparently did not receive any virus or not enough virus to produce immunity. If the injected pigs had no reactions, the control had no reactions.

The swine origin vaccine began to cause reactions in pigs quicker than the rabbit origin vaccines, and regained virulence quicker. This might be expected since anti-hog cholera serum is required with the use of this vaccine.

Field viruses have been obtained from more than 100 herds in which pigs were dying after vaccination with modified vaccine. Some of these viruses are quite virulent and will kill susceptible test pigs, some produce immunity without any visible reaction in susceptible pigs, and some produce reactions in test pigs but do not kill the pigs. There is no known way to determine if the virulent virus is of vaccine origin, but the nonvirulent virus must be of vaccine origin.

The action of virus V in this study is very similar to those viruses used from the vaccines. If vaccine 1B had been used in the field, and had passed through the herd so that it was obtained on the twentieth passage, then the passage results would have been comparable with virus V. It is, therefore, very probable that in this case the virus V was from the modified vaccine injected into the pigs on the farm.

The isolation of virus which produce slight or severe reactions in test pigs may be compared to the vaccines tested. If virus from vaccine III had been obtained from a herd after it had made nineteen passages the test pigs may have had a slight reaction. If this virus had made 29 passages the reaction in the test pigs may have been severe. The results strongly point out the need for anti-hog cholera serum to be properly used with live virus vaccines of rabbit origin to prevent eventual reactions and even death.

**SUMMARY**

Serial passages were made of four rabbit origin vaccines, one porcine origin vaccine, and one field virus. The first few passages of the rabbit origin vaccine produced immunity in the injected pigs and in the contact pigs with no visible reactions. All of the rabbit origin vaccines seemed to regain some virulence as evidenced by reactions in the test pigs. One vaccine did not kill any of the test pigs. The other three killed pigs in the higher passages.

The porcine origin vaccine produced reactions and immunity in the first passages, and death in the later passages.

A field virus isolated from sick pigs seemed to be similar to vaccine virus. All of the viruses passed from the injected pigs to the contact pigs.

No evidence of variant characteristics was observed in four of the viruses. One virus, when used with a test serum, caused a reaction in the pigs. They recovered and were immune.
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**TABLE 1**

Serial Passages of Modified Live Virus Vaccines
| 28 | SL+ | SL+ |
| 29 | SL+ | SV+ |
| 30 | SV+ | SV+ |
| 31 | SV+ | SV+ |
| 32 | SV+ | N+  |
| 33 | SV+ | N+  |
| 34 | SV+ | N+  |
| 35 | D   | SV+ |
| 36 | SV+ | SV+ |
| 37 | SL+ | SL+ |
| 38 | SL+ | SL+ |
| 39 | SL+ | SV+ |
| 40 | N+  | SV+ |
| 41 | SV+ | SV+ |
| 42 | SL+ | SL+ |
| 43 | N+  | N+  |
| 44 | N+  | N+  |
| 45 |     |     |

*1A; 1B—Commercial Lapine.
II; III—Origin Vaccine.
IV—Commercial Porcine Origin Vaccine.
V—Field Virus.
N—Normal—no reaction.
SV—Severe reaction.
SL—Slight reaction.
D—Died.
+—Immune to challenge 21st day.
——Susceptible after 21st day.
†—1. Pig one injected.
   2. Pig two injected.
   C. Contact control.
AD—Accidental death.
REFERENCES


A PRELIMINARY REPORT ON ATTEMPTS TO VACCINE PIGS FOLLOWING ANTI-HOG CHOLERA SERUM ALONE TREATMENT

H. W. DUNNE, D.V.M., Ph.D. and M. ALIBASOGLU, V.M., Dr.

One of the major problems confronting swine feeders today is the loss of swine purchased from community sales where anti-hog cholera serum alone had been administered. In some situations, these swine have been purchased at one sale or even from a private owner, given serum alone, and then submitted to another sales barn where the animal again may have received serum alone. The information regarding the treatments received prior to being admitted for sale and final vaccination on the farm may or may not have been known by the veterinarian. In approximately four to eight weeks, hog cholera may appear in these herds, many times in a chronic form. That is, individual cases appear slowly over an extended period of time but generally run a normal course of illness. Although a number of people have recognized the logic of anti-hog cholera serum blocking the production of active immunity, many have been willing to incriminate, without adequate proof, a variant virus against which the attenuated viruses were supposedly unable to develop an immunity.

It is not the purpose of this preliminary report to discredit the possibility of the occurrence of variant viruses nor the reversion of attenuated vaccines to pathogenicity, nor is it designed in any way to discredit vaccines, but rather to point out the relative importance of a basic principle of immunology which is neither new or original. Many years ago, Van Es (1) showed that pigs, given 1.72 cc. of serum per pound of body weight in simultaneous serum and virulent virus vaccination, did not develop an adequate active immunity against hog cholera. Doctor J. D. Ray (2), for years, has repeatedly advocated that, even using the virulent virus, good active immunity could not be obtained if the virus was administered earlier than seven days after the pigs had received a serum alone treatment. With these thoughts in mind a few preliminary experiments involving slightly more than a hundred pigs were conducted to get some indication of what might be expected if conditions arising at a community sale were duplicated experimentally.

Experimental

Swine used in these experiments were weaned pigs, 25 to 35 pounds in weight and from hog cholera susceptible sows except where indicated. The vaccine used was of rabbit origin and obtained fresh from the distributor.

From the Department of Veterinary Science, Pennsylvania State University, University Park. Authorized for publication as Journal Series Paper No. 2496 from the Pennsylvania Agricultural Experiment Station.

Acknowledgment for technical assistance is gratefully given to Dr. David C. Kradel, Robert B. Doty, and Dr. Robert J. Eberhart.
The serum was of commercial origin. Vaccinated swine were maintained under field conditions until the time of challenge when they were placed in isolation units. Groups were maintained intact to prevent undue fighting. All serum and vaccine were administered subcutaneously in the axillary region and/or in the flank. Upon challenge with one cc. of virulent hog cholera virus containing $10^6$ lethal doses, pigs showing a temperature rise to 104.6 or higher and a marked drop in total white blood cell count were considered sick.

**Experiment I Serum Block**

This was a preliminary experiment to learn if a possibility did exist that serum would interfere with vaccination and if a variation in the amount of virus in vaccination would make a difference. As will be noted in the chart, hogs weighing 100 to 125 pounds were pretreated with 40 cc. of anti-hog cholera serum which would be approximately 0.4 cc. per pound body weight. This was considered a minimum protective dose for this size animal. Four days later, which would approximate the time between purchase of pigs at a sale and subsequent vaccination, these hogs were given from one to 25 doses of vaccine and 15 cc. of anti-hog cholera serum. Four weeks later these animals were challenged subcutaneously with virulent hog cholera virus. Five of the six animals developed an abnormal temperature, showed a marked drop in total leukocytes, and were considered ill. Only two of the six animals died. The pigs that died had received one and five doses of vaccine respectively. Only the pig which received 25 doses of vaccine showed neither temperature rise nor a significant total leukocyte drop. The controls followed a typical course for hog cholera. With the low amount of serum used in the prevaccination treatment (0.4 cc./lb.), it appeared that increasing the dosage of virus in the vaccination might be important.

**Experiment II Serum Block**

This experiment was similar to the preceding one except that 25 to 30 lb. pigs were used and the serum dose was approximately one cc. per pound body weight. The interval between serum alone and vaccination was three days and the interval between vaccination and challenge was five weeks. Four pigs were in each group and vaccine dosage was one, 10, and 20 doses. Fifteen cubic centimeters of serum were given to each pig with the vaccine. Eight of the 12 pigs in all groups became ill and four died. Four remained well, showing no signs of illness. There was little difference in the groups receiving the various vaccine dosage although one might note that all four receiving just one dose of vaccine became ill whereas only two of each group receiving 10 and twenty doses of vaccine became ill.

**Experiment III Serum Block**

This was similar to the preceding experiments except that the pigs averaged 35 lbs., the serum dose was 30 cc. (approximately one cc. per pound body weight), and the interval between vaccination and challenge was six weeks.
There were four groups of four pigs each and the vaccine dose was one, five, 12, and 25 doses. Fourteen of the 16 having previous serum injections became ill upon challenge with virulent virus and seven of these died. There was no apparent correlation between vaccine dose and reaction to challenge.

**Experiment IV Double Serum Block**

In some instances pigs have passed through two sales barns a few days apart. Each time they received 30 cc. of anti-hog cholera serum. To test the effects of this on subsequent vaccination, three groups of 30 pound pigs, containing seven pigs each, were given two doses of 30 cc. of serum per pig three days apart. At intervals of three days, 10 days, and 20 days, one dose of vaccine without additional serum was injected subcutaneously. It was obvious from the results that the time interval made no difference. Upon challenge, all of the pigs but one became very ill and 17 of the 21 pigs died.

**Experiment V Double Vaccination**

The thought occurred that occasionally the actual use of both vaccine and serum might be repeated within a few days of each other. To make sure that this would have no effect upon the development of active immunity a group of 14 pigs weighing 30 pounds each was vaccinated twice, three days apart, using one dose of vaccine and 30 cc. of serum at each vaccination. There was no reaction in any of the pigs when challenged six weeks later.

**Experiment VI**

Twelve, five-week-old pigs from two sows, immunized against hog cholera with attenuated vaccines, were grouped into three categories with two pigs from each sow in each group. The first group received one dose of vaccine only per pig, the second group one dose of vaccine and 15 cc. of anti-hog cholera serum per pig, and the third group received 10 doses of vaccine and 15 cc. of serum per pig. One pig in the second group died from causes other than hog cholera before challenge. As the result of challenge all of the pigs in the group receiving one dose of vaccine plus anti-hog cholera serum became ill but recovered. Only two of the four pigs in group one which received vaccine alone became ill but one of these died. None of the pigs receiving 10 doses of vaccine became ill.

**DISCUSSION**

Although this is a preliminary report, there appears to be ample evidence that one cannot expect to actively immunize hogs which have received a protective dose of anti-hog cholera serum at least until a major portion, if not all, of the passive antibodies have been inactivated. Pigs vaccinated in the period during which some passive immunity still exists develop little active immunity and the majority are susceptible to hog cholera as soon as the passive immunity is dissipated. This fact is apparently more important when attenuated viruses are used than when virulent virus and serum were
given. Kovalenko (3) found that immune serum alone prior to vaccination with attenuated virus interfered with immunity production. Adequate active immunity was established when a second dose of vaccine without serum was given 10 days after the initial vaccination.

In contrast, Benner (4) commonly gave serum alone followed by virulent virus alone seven to 10 days later with reportedly excellent results. The work of Newberne et al. (5) using a lapinized virus indicated that vaccine given alone one week or more after administration of serum alone stimulated active immunity adequate to withstand challenge with virulent virus. Birch (6) routinely gave serum at three, six, and nine weeks and followed with vaccination (with virulent virus) at 12 weeks. None of the workers, however, indicated that vaccination would have been successful if it had been administered within seven days after serum alone was given. This suggests that the problem is not limited to the subsequent use of attenuated viruses but appears more pronounced following their usage than following the subsequent use of virulent virus. This does not present an unsurmountable obstacle to the use of the attenuated vaccine. If all pigs passing through community sales were required to be vaccinated with both vaccine and serum, the problem would not exist.

The ratio of the number of antibodies in the serum given alone to virus particles in the vaccine may be important. In most of the experimental situations reported here, however, the upper limit of numbers of virus particles apparently was not reached. Actually, the ratio of the prevaccination serum dose to pig weight appeared to have more effect than the variation of the dose of vaccine.

Pigs from sows vaccinated with lapinized virus apparently can be actively immunized against hog cholera at five weeks with only slight interference from maternal antibodies. It has been shown by Kalikan et al. (7) and Smith and King (8) that such pigs had low maternal antibody absorption as compared to pigs from sows immunized with virulent virus. The latter pigs at an early age definitely show interference with active immunization even when virulent virus and serum are given (Cahill 9).

**SUMMARY AND CONCLUSIONS**

It is again brought out that active immunity cannot be established satisfactorily in the presence of excessive circulating antibodies. In view of this additional evidence which is presented to revive thinking on an age-old problem, it seems imperative that consideration be given to some of the regulations regarding the treatment of swine passing through community sales. Since a major problem is associated with attempts to actively immunize swine previously given serum alone, it is strongly suggested that all swine passing through community sales be required to receive both vaccine and serum. Such a regulation, if followed in all states, would largely eliminate one of the greatest headaches of the feeder industry today.
REFERENCES


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REPORT OF COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


Much information has been presented to your Committee on the apparent inability of modified live virus vaccine to establish permanent immunity in swine as evidenced by many so called “breaks” that have been occurring recently throughout the country. The Committee is of the opinion, however, that based upon investigations that have been conducted in conjunction with these outbreaks, in a majority of the cases non-vaccinated swine have been involved. Your Committee realizes that modified live virus vaccines will not confer permanent immunity in all swine treated; however, it is felt that immunity failures have been exaggerated far out of proportion and have instilled a lack of confidence in the use of modified live viruses.

Reports have also been heard that the improper usage of modified vaccine may be widespread in given sections of the country. Such practices range from the administration of half doses of vaccine in the treatment of swine to the use of inadequate amounts of serum in conjunction with vaccine. It must be recognized that the use of modified live viruses will not confer the desired immunity in swine if label instructions are not adhered to and sound judgment and proper techniques are not employed in the use of these modified live viruses. Failure to follow such practices not only results in the inability to establish immunity, but also tends to confuse the farmer and thus reduce his confidence in the use of vaccines.

Based on information received from results obtained in the Florida pilot-test program with reference to the degree of immunity conferred in relation to the expiration date of modified live virus vaccines, your Committee wishes to recommend to the Biologics Inspection Section of the United States Department of Agriculture that consideration be given to shortening the expiration dates of these products; and further, that such expiration dates should be established from the date of production of the products rather than from the date upon which tests are completed.

Widespread reports have rather conclusively established that modified live virus vaccines have failed to produce firm immunity, particularly in market swine, which have previously received in many cases multiple quantities of serum alone as these swine move from market to market. Your Committee recognizes that serum interference constitutes a problem of great magnitude in the eventual establishment of permanent immunity to hog cholera, and has seriously considered the current trends in the marketing of swine with respect to cholera immunization practices. The use of serum alone in vaccinating
swine for immediate movement dates back to the era when virulent virus was generally accepted as the only product for permanent immunization. Recognizing the inherent hazards of disease spread from pigs recently vaccinated with virulent virus, serum alone was used as an alternative. It is realized that while there may be stress factors involved with in-transit pigs which have recently received modified live virus vaccine and serum, the losses which would occur from such stress would be minimal in comparison to the losses sustained from the inability to later produce permanent immunity as the result of serum interference. Your Committee recommends to the several states that consideration be given to the discontinuance of the serum alone method of treatment at sales markets, and in lieu thereof to follow the practice of vaccinating swine simultaneously with modified live virus vaccine and adequate amounts of serum, except in those cases where, based on scientific data, the use of the vaccine is contra-indicated. It is further recommended that the United States Department of Agriculture amend its existing hog cholera regulations to conform to the above recommendation on all interstate movements of swine.

Your Committee wishes to reemphasize the need for continued research in the improvement of immunizing agents for the protection of the nation’s swine industry from the ravages of cholera; however, it is felt that the advantages that have been demonstrated through the use of the modified live virus vaccines, while not the ultimate, far exceed those obtained with virulent virus, particularly in the light of the hazards that exist through the use of virulent virus. A positive, rather than a compromising approach must be taken by industry leaders with respect to stringent restrictions on the use of virulent virus if the nation is ever to eradicate hog cholera.

In furtherance of this aim, and as evidenced by the fact that 32 states have now prohibited the use of virulent hog cholera virus, the Committee feels that restrictions should be placed on the interstate movement of virulent hog cholera virus, and to this end recommends that except for research and serum and vaccine production, the movement of virulent hog cholera virus between states be prohibited by the United States Department of Agriculture.

It is gratifying to note that during the past year, Livestock Conservation, Incorporated, acting upon previous recommendations of this Committee, prompted action on the part of the Federal Extension Service and the Animal Disease Eradication Division of the United States Department of Agriculture to hold regional meetings between industry representatives and livestock sanitary officials for the purpose of establishing hog cholera eradication programs in the several states. Four regional meetings have been held throughout the country since April; however, the attendance at these meetings by industry representatives has been disappointing. Reports received indicate that a few states have taken action toward implementing statewide eradication programs. Observations in these states demonstrate that if such a program is to be successful its activities must be directed to the grass roots elements of the industry so as to encourage greater participation by the swine producer, who is the direct beneficiary.
Your Committee strongly urges that greater impetus be given to engendering interest in hog cholera eradication on local levels, with particular emphasis directed toward more active participation by the swine producer without whose full support such a program cannot succeed.

In the adoption of an over-all hog cholera eradication program, it is recognized that one of the deterents to the success of such a program is the lack of a rapid conclusive diagnostic test. Because of the importance of such a test in the conduct of hog cholera eradication programs, the Committee recommends that a crash program of research be directed towards the development of a rapid accurate method of diagnosing hog cholera.

In order to coordinate on a national level statewide hog cholera eradication programs and to implement uniform rules and regulations in dealing with the disease of hog cholera as approved by the United States Livestock Sanitary Association, your Committee recommends the establishment of a Swine Disease Section within the United States Department of Agriculture.

In previous reports of this Committee, recommendations have been made that increased backlogs of anti-hog cholera serum should be maintained for use in the event of cholera outbreaks throughout the country. These recommendations have heretofore gone unheeded, thus creating a condition which could have proven disastrous had widespread hog cholera occurred during these recent years. It must be recognized that it is economically unsound for producers to maintain such quantities of serum as would be needed in the event of widespread hog cholera epidemics. However, your Committee feels that this issue is of such importance to the industry that it hereby recommends that the United States Department of Agriculture give consideration to implementing a system whereby it may maintain stockpiles of serum which could be used in the event of a national emergency.
THE IDENTIFICATION AND TYPING OF VESICULAR EXANTHEMA BY COMPLEMENT FIXATION AND AGAR DIFFUSION TESTS

T. L. BARBER, D.V.M., W. M. MOULTON, V.M.D., and S. S. STONE, PH.D.*

INTRODUCTION

Swine are susceptible to three vesicular diseases: foot-and-mouth disease (FMD), vesicular stomatitis (VS), and vesicular exanthema (VE), all of which must be considered in differential diagnosis (12). After eradication of VE from the United States, responsibility for diagnosis of the disease was transferred to the Plum Island Animal Disease Laboratory (PIADL). The following describes a complement fixation (CF) and an agar diffusion (AD) procedure for identifying and typing VE virus and antiserums.

MATERIALS AND METHODS

Swine of mixed breeds and weights were used for virus and serum production.

Viruses: Eleven types of VE virus, obtained from the National Animal Disease Laboratory (NADL), Beltsville, Maryland, were passaged in pigs. Vesicle coverings from infected feet were used in CF studies as antigen.

Antiserums: VE antiserums were produced by Virological Investigations, NADL, subsequent to 1954, except for type E54. This serum was produced by inoculating swine intradermally in four feet with VE virus, type E54, and bleeding the animals at intervals. Serums were stored at -40°C.

Complement Fixation Test

Antigen for the CF test was prepared from swine foot epithelium by mincing and grinding with an abrasive in mortar and pestle. The material was diluted 1:10 in veronal buffered saline during grinding. The suspension was clarified by centrifuging twice at 10,000 X G for 30 minutes with one intermediate freezing and thawing and was stored frozen in small aliquots. Just prior to use in the CF test, this material was thawed and centrifuged 2,500 X G for 15 minutes. Tissues were removed from normal pigs’ feet and treated the same way as above to be used as normal controls in the CF test.

Antiserums: Antiserums were diluted and heat-inactivated at 56°C for 30 minutes.

Diluent: Veronal buffered saline (VBS), pH 7.2, containing 0.1 percent gelatin was used as diluent (10).

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Complement: Pooled fresh guinea pig serum stored at \(-40^\circ\text{C}\) was used as complement (\(C'\)).

Indicator System: Two percent sheep erythrocytes were optimally sensitized with equal volume of commercial hemolysin (10).

Complement Fixation Procedure: The complement fixation procedure was essentially the same as that used at Plum Island Animal Disease Laboratory (PIADL) for typing FMD and VS antigens and antiserums (3). Constant dilutions of antigen and serum and varying dilutions of \(C'\) were used. Optimal dilutions were found by titration of various ratios of serum, antigen, and \(C'\). A stock dilution of \(C'\), usually 1:40, was prepared from which subdilutions were made, each containing 50 percent more \(C'\) than the preceding: four, six, nine, 13, 20, 30, 45, 67 and 100 percent. The two lower subdilutions were used only to show the degree of procomplementary activity of swine serum but are not essential for the determination of \(C'\) fixed. Volume deliveries per tube were as follows: antigen 0.1 ml., serum 0.2 ml., and complement 0.5 ml. Control rows were run for each antigen and serum replacing the deleted component with a like volume of diluent.

Following the fixation period of 18 hours at 4 to 6\(^\circ\text{C}\), 0.5 ml. of indicator system was added to each tube and the test incubated 30 minutes at 37\(^\circ\text{C}\). To attain the minimum volume required for spectrophotometric readings, 0.7 ml. VBS was added to all tubes before they were centrifuged at 1,000 \(X\) \(G\) for five minutes. The degree of hemolysis was determined spectrophotometrically and the percentage of hemolysis was calculated.

![Figure 1](attachment:image)

**Figure 1.** Complement fixed by different ratios of VE antigen and antiserum.
The logarithm of the unit of $C'$ fixed was calculated by plotting on arithmetic graph paper. The ordinate was the logarithm of the percentage of stock $C'$ and the abscissa was the percentage of hemolysis. Only points between 20 and 80 percent need be plotted (1), (7). The 50 percent endpoint was determined graphically and fixation expressed as the difference between the logarithmic value for antigen and that of the homologous system.

**Agar Diffusion Test**

The agar double-diffusion (AD) technique (4), using borate buffer pH 8.6, ionic strength 0.05, was used to demonstrate specific antibody.

Antigen for the agar diffusion test was prepared using primary pig kidney monolayer cultures (8). Forty-eight hours after inoculation, the fluid and cellular debris was centrifuged at 10,000 X G for 30 minutes. The pellicle was reconstituted with diluent to four percent of its original volume. This material was frozen at least once before use. Uninoculated tissue cultures were treated in a similar manner for controls.

Serums were heat-inactivated at 56°C for 30 minutes and used undiluted.

**RESULTS**

Figure 1 shows the complement fixed by varying ratios of antigen and type E convalescent serum. Blood was withdrawn at 37 days post-inoculation (DPI). Normal tissue was tested at 1:10 and normal serum at 1:20.

Antigen at 1:10 in the presence of lower antibody levels, 1:40, 1:50, and 1:60, showed a prozone effect.

![Figure 2](image-url)  
**Figure 2.** Complement fixing antibody response in two age groups of swine after inoculation with VE type E.
There was no CF when normal serums and tissues were substituted for convalescent serum or infected tissue.

Figure 2 illustrates the CF antibody response of four six-month-old pigs and four six-week-old pigs inoculated with VE type E virus. Titers of the individuals of each group were averaged and plotted. Convalescent serum antibody from all pigs fixed complement in excess of logarithm 0.4. The older group gave no demonstrable CF antibody at eight DPI, and individuals reached peaks between 15 and 29 days. Bleedings were terminated at 37 DPI. There was greater individual variation within serums of the younger group. Three gave no antibody response at seven days, a rising antibody titer at 14 days with maximum fixation between 19 and 29 DPI. One pig gave no CF response until 29 days and remained at a high level through 51 DPI. Three pigs of this group were bled through 102 DPI at which time a low level CF antibody persisted.

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<th>H</th>
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* Logarithm C' Fixed.
— Not Tested.

CF antigens were prepared from all 11 virus types for testing of VE antiserum pools received from NADL. Fixation results of the available convalescent swine serum pools are given in Table I. With types B, D, E, G, H, I, and K, the homologous serum could be identified. Three additional types (A, F, J) of convalescent serum failed to show adequate CF antibody.

Swine that were infected and hyperimmunized by reinoculation with the same virus type produced serums that were not type specific but gave significant CF with most or all VE virus types. Three types of hyperimmune swine antiserum which fixed C' in the presence of all 11 VE types are shown in Table II.
IDENTIFICATION OF VESICULAR EXANTHEMA

TABLE II

C' Fixation Results with VE Hyperimmune Swine Serums with Eleven VE Virus Types

<table>
<thead>
<tr>
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<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
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<td>.73</td>
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<td>.75</td>
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</table>

* Logarithm C' Fixed.

Holbrook showed that these convalescent and hyperimmune serums have type specific neutralizing antibodies (8).

All VE serums were tested against FMD virus types C and Asia 1 and VS virus types New Jersey and Indiana with no evidence of C' fixation.

The VE antigens were tested with all seven types of FMD hyperimmune guinea pig serums and the two types of VS antiserums. There was no C' fixation with the FMD or New Jersey VS antiserums. Indiana VS convalescent swine serum fixed limited amounts of C' but not sufficiently to interfere with the specificity of the test.

TABLE III

AD Results with VE Convalescent and Hyperimmune Swine Serum and 8 VE Antigens Obtained from Tissue Culture

<table>
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<th>D</th>
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<th>G</th>
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<th>I</th>
<th>J</th>
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Hyperimmune

B247  +  +  +  +  +  +  ±  +  +  +  +  +
B2751  +  +  +  +  +  +  +  +  +  +  +
C      +  +  +  +  +  +  +  +  +  +  +
J II   +  +  +  +  +  +  +  +  +  +  +
J III  +  +  +  +  +  +  +  +  +  +  +

Normal Serum

|   |   |   |   |   |   |   |   |   |   |   |   |           |   |   |

+ Clear Crisp Line.
± Diffuse Line.
— No Visible Line.

Results of AD tests are shown in Table III and are similar to those obtained with CF.
Eleven types of VE virus were isolated between 1944 and 1956 and identified by neutralization tests in swine. Tissue culture techniques make it possible to type VE virus more conveniently (2).

All types did not propagate readily in tissue culture (TC) from field isolates but all have been adapted to swine kidney cultures. Tissue culture neutralization can be utilized for detecting antibodies in the sera of swine infected with one of the TC-adapted types. The epizootiology of VE indicates the ease of mutation by the virus with the possibility that new virus types may appear should the disease recur (9).

The desirability of having a serological test capable of identifying all VE types with a single heterologous antibody source has been stressed (8).

VE convalescent and hyperimmune swine serums prepared by reinoculation with a single virus type, produced type specific neutralizing antibody (8). As measured by the CF and AD procedures described only convalescent serums retain their type specificity. Hyperimmune serums were not type specific and reacted with heterologous VE antigens. This characteristic may be related to variance in specificity in early and late antibody as demonstrated by Brown and Graves, showing changes in type specificity in the serums of cattle recovering from FMD (5). However, it is more likely that the lack of type specificity may be due to the method of immunization. Burnet states: "In general the response to a single injection is to produce more specific antibody than is found when the animal receives a series of repeated injections" (6).

The C' dilution method of determining the presence of antigens and antiserums offers advantages by measuring each of the factors affecting C' and its fixation (11). This is especially desirable in testing tissue samples submitted from the field as antigen sources. It also permits the use of swine serums for CF antibodies without interference from its unpredictable procomplementary activity. Under laboratory conditions, the optimal antigen dilution was between 1:30 and 1:40. With field samples, it may be necessary to use the antigen in lower dilutions within the limits of the zone phenomena shown in Figure 1.

While the zone phenomenon may be an example of C' being fixed by varying antigen-antibody proportions, it may also be associated with the presence of inhibitory factors in tissue antigen. As the inhibitory substances are diluted, additional C' is fixed (13).

SUMMARY

Vesicular exanthema-infected swine tissues and tissue cultures along with convalescent and hyperimmune swine serums transferred to the Plum Island Animal Disease Laboratory were assayed by complement fixation and agar diffusion tests.

The procomplementary activity of swine serum did not interfere with interpretations of results with the complement fixation procedure described.
The rise and fall of complement fixing antibody in convalescent swine serum was shown.

Seven virus types were identified by complement fixation using convalescent swine sera and infected tissue antigen. Comparable results were obtained using agar diffusion with tissue culture antigens.

Two hyperimmune swine sera provided heterotypic antibody sources that made possible the screening of the existing types of vesicular exanthema from vesicular stomatitis and foot-and-mouth disease by complement fixation and agar diffusion tests.

REFERENCES


OUTBREAKS OF VESICULAR STOMATITIS IN OKLAHOMA AND TEXAS

N. L. MEYER, D.V.M.; W. M. MOULTON, V.M.D.
E. W. JENNEY, D.V.M., B.S.; R. J. RODGERS, D.V.M.*

Vesicular stomatitis (VS), vesicular exanthema (VE) and foot-and-mouth disease (FMD) are usually characterized by the formation of vesicular lesions in the mouth and/or feet and/or teats of affected animals. Even the most expert diagnostician cannot distinguish between these three diseases by clinical examination (1). Fortunately VE was brought under control and its complete eradication was announced by the Secretary of Agriculture in October 1959. The last North American outbreak of FMD occurred in Mexico in 1953. Only vesicular stomatitis remains a problem in the United States. Since VS cannot be distinguished clinically from VE and FMD every outbreak of a vesicular condition must be differentially diagnosed by animal inoculation or laboratory tests. Every vesicular disease outbreak presents a challenge to the veterinary profession and particularly to the livestock disease control official. Rapid diagnosis is essential so that if VE or FMD should reappear officials may act quickly to accomplish eradication at minimum cost.

VS remains a problem because of the lack of sufficient knowledge concerning the disease to accomplish effective control and eradication. It is hoped that the presentation of information concerning investigations of epizootic and enzootic VS will stimulate interest in further investigation and research.

Previous reports have shown that VS appears seasonally in enzootic areas. For the purpose of this report an enzootic area is defined as an area in which 1. a high percentage of the cattle and swine carry VS virus neutralizing antibodies, 2. neutralizing antibodies are found in wild animals, and 3. VS is diagnosed serologically for a number of successive years (2). The coastal plain area of southeastern United States and some areas of Mexico are considered enzootic. It now appears that there may be other enzootic areas.

In contrast, epizootic VS is characterized by 1. irregular occurrence, 2. high incidence of lesions in affected herds, 3. rapid spread, and 4. the conspicuous absence of infection in swine.

METHODS OF INVESTIGATION

When a livestock disease condition is reported as being suggestive of a vesicular disease, an immediate investigation is conducted. State and federal animal disease control officials assign a Veterinary Diagnostician to make an examination of the affected animals. These Diagnosticians have been specially trained in the differential diagnosis of vesicular diseases and other diseases with which they might be confused. When examination indicates that a vesicular disease does exist or may have existed, tissue and/or serum samples are collected for animal passage or serological laboratory diagnosis.

* Animal Disease Eradication Division, Agricultural Research Service.

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LABORATORY DIAGNOSIS

Epithelium and fluid from recently-formed vesicles are the materials of choice for laboratory diagnosis. If vesicular material is not available, the affected animals are bled to attempt the demonstration of complement fixing or serum neutralizing antibodies in convalescent serums. Antibodies usually appear five to 10 days post infection; however, the optimum time for the collection of sera is 21 days post infection (3, 4). When material from a vesicular lesion is available, it is prepared as antigen for a complement-fixation (CF) test against antiseraums of the seven known types of FMD and two types of VS.

Serums, submitted for diagnosis, which give negative results to the complement-fixation test for VS are tested by a serum neutralization test in embryonating chicken eggs or in tissue culture. Complement-fixing antibodies to VS can be demonstrated for only a few months following clinical infection, but serum neutralizing antibodies persist for several years. Paired serum samples, with the first collected at the time of the initial investigation and the second after about three weeks convalescence, are often necessary to establish a diagnosis, especially in enzootic areas where lesions may be comparatively mild and the percentage of clinically affected animals rather low.

To be of value, information concerning the seasonal occurrence of VS must be accurate and as complete as possible and is, therefore, dependent on careful surveillance and accurate diagnosis. Observations became more critical in 1952 with the outbreak of VE and the urgency of providing differential diagnosis for FMD, VS and VE. Laboratory information concerning the incidence of VS from 1952 to 1960 is summarized. (Tables I and II.) Only laboratory-confirmed diagnoses are shown. In the interpretation of this information we must acknowledge that the true incidence of infection is not represented. Laboratory tests are conducted each time the disease appears in an area. In epizootic VS hundreds of cases may appear over night making individual herd or individual animal diagnosis impractical if not impossible. If a change is noted in the clinical or epizootiological characteristics of an outbreak, additional samples are submitted for a confirmatory diagnosis.

EPIZOOTIC VESICULAR STOMATITIS IN OKLAHOMA IN 1957

The appearance of VS in the “Little River” area of Oklahoma-Arkansas in 1957 provided an opportunity to study some of the characteristics of epizootic VS. Personnel to conduct the investigation was made available by the state-federal animal disease control officials of Oklahoma and Arkansas. They are to be commended for their complete cooperation and dawn-to-dusk effort, seven days a week until the outbreak subsided.

The survey disclosed that the first clinical case of VS appeared about June 13, 1957 and the last known case about July 7, 1957, with infection most prevalent in the flood areas of the Little River and its tributaries. The Little River is located in southeast Oklahoma primarily in McCurtain County and extends into Sevier and Little River counties in Arkansas.
TABLE 1

*Initial Laboratory Diagnoses of Vesicular Stomatitis By States and Years*

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* New Jersey type except as indicated.
### TABLE II

Laboratory Diagnoses of Vesicular Stomatitis by Species in Enzootic and Other Areas

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<th>Year</th>
<th>Species</th>
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<th>Other Areas (No. of Premises)</th>
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</table>

(as of August 25, 1960)

* Enzootic areas regarded as Georgia, South Carolina, North Carolina, and Louisiana.

At the periphery of the infected area the incidence of farm infection was very low, increasing in concentration toward the Little River with an incidence of about 95 percent at the center of the infected area. On infected premises, morbidity varied from less than five percent to more than 75 percent of susceptible animals showing clinical signs. Mortality was negligible and in all cases was attributed to secondary infection of untreated lesions.

Infection was found on 372 premises with 14,268 cattle. Clinical symptoms were seen in 1,428 (10 percent). Mouth lesions only were visible in 857 (61 percent). Teat lesions only were apparent on 191 (13 percent). Mouth
and teat lesions were evident in 378 (26 percent). The condition was diagnosed in the laboratory as New Jersey type VS.

There were 484 equines on 194 of the total infected premises. Lesions were present on 73 (15 percent); lesions of the mouth only in 40 (55 percent); foot lesions only in 17 (23 percent); while 16 (22 percent) disclosed foot and mouth lesions. The foot lesions appeared as swelling of the tissue in the coronary region and the area above the frog with separation of the hoof and underlying tissue and oozing of serum from the frog. A severe lameness resulted.

There were 1,054 swine on 92 of the 372 infected premises. Seven swine showed lesions suggestive of VS, but laboratory tests did not identify the condition as a vesicular disease.

The primary source of infection was not determined; however, the almost simultaneous appearance of infection throughout the area indicates that transmission was other than by physical contact with infected animals. This investigation leaves us with at least two fundamental questions: 1. “Where did the disease originate—Does a reservoir exist and what is it?”, and 2. “How was the disease transmitted?” Some of the factors which may be important in attempting to answer these questions were investigated.

**Topography:** The land slopes toward the south and is drained by Mountain Fork River, Little River and Clover Creek in Oklahoma; Rolling Fork, Bear Creek, and Pond Creek in Arkansas; and their tributaries. These streams, with alluvial bottoms, had been subjected to serious overflows about 30 days prior to the outbreak of VS. Most of the northern part of the area is mountainous, while the southern part is rough, rolling land with numerous creek bottoms of flat land. It was in these creek bottoms and second creek bottoms that the highest incidence of infection was observed.

**Soil Types:** Three distinct soil types were identified within the infected area: 1. Bottom-land soils in overflow areas which flood frequently and are poorly drained; second bottoms or benches; 2. sandy upland soils with clay or sandy clay subsoils; 3. rough, stony lands on the steeper slopes where the soil is shallow and covered mainly by forest comprises the northern part of the affected area.

The region affected is the upper limit of what is known as the coastal plain area and includes soil types identical to those found in the Carolinas and Georgia, where VS occurs enzootically.

**Ground Cover:** Native tree growth is pine, much of which has been marketed. Other trees are red oak, post oak, blackjack oak, hickory, gum, and cedar. Native grasses include little and big bluestems, switchgrass, and Indian grass.

**Climate:** The average annual temperature is 64° F., with the average high of 94.6° F. occurring in August and an average low of 32.8° F. in January. Generally the last killing frost in the spring occurs the third week of March and the first killing frost in the fall the second week of November.

The average rainfall is 54 inches with 75 percent received during the growing season.
VESICULAR STOMATITIS IN OKLAHOMA AND TEXAS

Annual rainfall: 1952 48½ inches
1953 43 inches
1954 40 inches
1955 43 inches
1956 30 inches
1957 Jan. 4.91 inches
Feb. 3.78 inches
March 8.32 inches
April 14.34 inches 48 inches
May 12.42 inches
June 4.77 inches

Insect Population: Information available on the distribution of mosquitoes in Oklahoma indicating types of breeding water preferred and the distribution of larvae and adults by months, incriminates several species. The role of Culicoides spp. must also be considered. Tabanids and other horseflies did not fit the epizootiological pattern of this outbreak.

Fauna: Of the annual deer kill in Oklahoma, 40 percent are taken from McCurtain County. Other species present include: raccoons and opossum in abundance, some coyotes, skunk, bobcat and squirrels.

Economics: Infected dairy herds experienced a reduction of milk production to an estimated 30 percent of normal, which gradually returned to nearly full production after 20-25 days. Many cattle of both dairy and beef breeds developed mastitis after the appearance of teat lesions.

In range herds, affected animals lost from 75-450 pounds with an estimated average loss of 130 pounds per animal. At contemporary market quotations for standard and commercial grades, this was an average of $20/affected animal. We consider this a conservative minimum reflecting immediate loss three weeks after the appearance of infection. Factors such as time required to replace weight loss, impairment of function from secondary infection, stunting of calves with a decrease in value as registered replacements, time required for treatment, drug and veterinary expense, and restriction of commerce, were not included in the estimate of loss.

EPIZOOTIC VESICULAR STOMATITIS IN TEXAS IN 1959

Vesicular stomatitis was reported in an enzootic area in southern Mexico in November 1958 and later as far north in Mexico as a point ten miles west of the Rio Grande River opposite Starr County, Texas. The heaviest infection in Texas occurred in an adjacent area across the Rio Grande River between Roma and Rio Grande City.

The first VS in Texas during 1959 was reported May 4 near Mentone which is in Loving County in western Texas. The infection was limited to two horses on one ranch. Inspection of dairy herds in Loving, Reeves and Pecos counties revealed no additional infection.
The next evidence of VS was found on May 6 in horses used in patrolling the Rio Grande River in Webb County near Laredo and on May 20 in cattle and horses near Del Rio in Val Verde County. Between May 20 and June 9, tissue from bovine vesicular lesions was submitted for laboratory diagnosis from Starr, Zapata and Dimmit counties. All were positive for New Jersey type VS.

On June 16 a ranch near Carrizo Springs, also in Dimmit County, was inspected. Infection had appeared in horses first and later became apparent in cattle. Vesicular material from the tongue and teats of several cattle was submitted to the laboratory and found to be positive to New Jersey type vesicular stomatitis. This ranch was located on the shores of an irrigation reservoir where mosquitoes, flies and gnats were abundant. Farms in the area were heavily covered with brush, weeds and grasses and many were irrigated. On infected farms morbidity was usually less than five percent.

Only two infected premises were reported in the area further south between Rio Grande City and Grulla where the farms are large with open country and investigations as far north as Kerr and Comal counties revealed no clinical VS.

Infection was identified on 115 premises involving cattle and 24 involving horses and mules. The 115 infected premises had a total of 4,157 cattle and of that number 451 (10.8 percent) were affected. The morbidity in infected herds varied from less than one per cent in Kinney and Val Verde counties to 16 percent in Starr County.

Of the 451 infected cattle, 111 (24.6 percent) showed mouth lesions, 133 (29.4 percent) showed udder lesions and 207 (46 percent) showed both mouth and udder lesions. There were no livestock deaths attributed to VS.

Infected horses and mules were found on 24 premises. Of 197 horses and mules on these premises vesicular lesions were seen in 37 (19 percent). No foot lesions or lameness was reported in infected horses and cattle and no infected swine were found.

Although VS was also present across the river in Mexico the primary source of infection was not determined and the simultaneous appearance in scattered areas along the Rio Grande indicates the probability of transmission by some means other than infected animals or mechanical transmission.

We are again faced with the questions: 1. "Where did the disease originate—Does a reservoir exist and if so what is it?" 2. "How was the disease transmitted?"

*Topography:* The region abounds in lakes, streams, and canals. Heavy rains following a dry spell had raised the water level, filled ponds and tanks, and covered low-lying pastures. It was here that infection was concentrated. Livestock in upland pastures and well-drained fields remained free of the disease.

*Fauna:* Deer, raccoon, bobcats and opossum populate the area along the Rio Grande River.

Many crows, blackbirds, sparrows, and some wild ducks were seen on infected premises before and during disease outbreaks.
Insect Population: Prior to, and during the outbreak, large numbers of biting gnats, deer flies, horseflies and mosquitoes were reported.

Ground Cover: The heaviest infection was found in areas where trees, weeds and brush were exceptionally luxuriant. It should be noted, however, that livestock population is also more concentrated in such areas.

Public Health Aspects: Several cases of tonsillitis in children, with high fever, sore tongue, vesicles on tongue and lips, and muscular pains and discomfort were reported by medical authorities. These children lived near the focus of animal infection.

In another case, at the peak of the outbreak, a 35-year-old rancher contracted an influenza-like condition with tonsillitis, high fever, and body pains that lasted for six days.

Vesicular lesions had been found in many of his cattle and horses, and vesicular material taken from teat and mouth lesions had proven positive for New Jersey type vesicular stomatitis. Two blood samples were taken. The initial sample taken three days after onset of illness was negative for antibodies to New Jersey type vesicular stomatitis. The second sample taken 14 days later yielded a high titre of VS virus neutralizing antibodies.

Economics: Heavy weight losses were reported in some cattle. Other owners reported cattle off feed for two to three days, but no weight losses. Horses seemed to be more noticeably affected and experienced greater difficulty in eating.

CONCLUSIONS

Additional study of epizootic and enzootic VS is necessary before we can determine and evaluate the factors significant to eventual eradication. It is evident that investigations conducted to date were not sufficiently comprehensive to supply the “key” information upon which to base an effective eradication program.

A review of the Oklahoma 1957 and Texas 1959 outbreaks of VS indicated:

1. Initial infection is not the result of the introduction of clinically infected domestic animals.
2. Initial infection and early subsequent infection usually occurs in the bottom lands or flood areas of rivers and streams.
3. Infection appears after flooding of areas normally covered with vegetation.
4. Spread of infection is rapid and does not conform to the patterns of traffic by humans or domestic animals.
5. The duration of an outbreak within a given area does not exceed six weeks.
6. Swine have not become infected in epizootics of VS in contrast to the high morbidity in swine within enzootic areas.

These conclusions will no doubt be changed and amended as additional information is developed.
REFERENCES


REPORT OF THE COMMITTEE ON VESICULAR DISEASES

F. J. MULHERN, Chairman, Falls Church, Virginia; J. J. CALLIS, Greenport, L. I., New York; A. A. ERDMANN, Madison, Wisconsin; R. P. HANSON, Madison, Wisconsin; L. KARSTAD, Waycross, Georgia; S. H. MADIN, Berkeley, California; N. L. MEYER, Alexandria, Virginia; L. O. MOTT, Beltsville, Maryland; J. TRAUM, Berkeley, California.

INCIDENCE OF VESICULAR DISEASES IN THE UNITED STATES

A year ago October 22, 1959, the secretary of agriculture announced the eradication of vesicular exanthema of swine from this country and so far as is known from the world.

This situation should not be a signal for relaxation of effort or responsibility on our part relative to continued vigilance and effort to add to our knowledge of this disease.

With the reduced number of personnel engaged in inspection of garbage feeding premises it is difficult to get the desired inspection of such facilities. The Vesicular Disease Committee Report for 1959 urged that the inspection of garbage feeding premises be assigned to personnel currently on state and federal rolls so that such premises will receive regular inspection. We re-emphasize the need for adjusting work loads and assignments to accomplish regular inspection of all garbage feeding premises.

The Committee wishes to again emphasize the importance of garbage cooking to assist in the control of other diseases of swine such as hog cholera, trichinosis, and erysipelas. An established program of garbage cooking is essential in preventing the introduction of foreign animal diseases into this country. It should be remembered that the outbreaks of FMD in California in 1924-29 have been linked with the feeding of raw garbage from ships that had provisioned in foreign countries where FMD was indigenous. There is strong indication that the most extensive outbreak of this disease in this country 1914-1916 was introduced by the feeding of raw garbage.

A suspected vesicular condition affecting the mouth, feet and udder of cattle was reported in southern Oklahoma and northern Texas during August and September of this year. Investigation revealed approximately 55 herds located in Bryant county, Oklahoma, and Fannin, Grayson and Cooke counties in Texas were affected. Sera and tissue samples submitted from these cases were negative to serological tests for vesicular viruses. Lesions were described by an experienced diagnostician as similar to those sometimes observed in certain stages of VS. The importance of thorough investigations of each condition affecting the feet and mouth of animals cannot be over-emphasized. Although the condition found in Oklahoma and Texas proved negative to the tests for vesicular virus and laboratory investigation continues to arrive at a definitive diagnosis is being continued.
STATUS OF PRESENT KNOWLEDGE OF VESICULAR STOMATITIS

Vesicular stomatitis appears to be limited to the Western Hemisphere where it is enzootic on the coastal plain of the region extending from the Carolinas southward around the Gulf of Mexico and the Caribbean Sea into South America involving Venezuela and Colombia. At intervals epizootics occur south to Peru and Argentina in South America and north into Canada in North America. In the past the disease has been carried to other continents especially in time of war or when there has been a large movement of horses from the Americas. Except in those areas where the disease is enzootic, vesicular stomatitis does not appear to be self-perpetuating.

In the United States the disease appears each spring and disappears in the fall, while in Mexico infection can be found any month of the year. The incidence of disease appears to correspond closely with the insect season. It is significant that numerous investigators in widely separated regions have observed the prevalence of the disease in animals on wooded and/or wet pastures and less frequently in open dry pastures and stables. During the spread of the disease it follows much the same pattern each time. Often it is found associated with a river or stream. Known infections may be five or 10 miles apart. Quarantines have not been effective against the disease.

The techniques for studying the vesicular stomatitis virus are well developed. The virus can be isolated readily in most tissue culture systems with a readily noticed cytopathogenic effect and can also be isolated in embryonating eggs or laboratory animals. The diagnostic tests for this disease include in the complement-fixation, the serum-neutralization, the agar diffusion, and other tests. Guinea pigs, chickens, mice, rats, hamsters, chinchillas, ferrets, and other small animals have been used successfully in the identification and typing of vesicular stomatitis.

While certain facts are known about the epizootiology of vesicular stomatitis, a considerable portion of the story is yet to be learned. The disease has a restricted geographical distribution, a seasonal appearance, and a host-range that is known to include domestic animals, wild animals, and man. The reservoir of infection and mode of transmission have not been established.

VS (N. J. type) has been diagnosed in four states during 1960—North Carolina, South Carolina, Louisiana and Texas. Only a few cases were reported in these states. The comparatively small number of reports is believed to be an indication of a lower incidence of the disease.

RESEARCH ON VESICULAR DISEASES

Vesicular Exanthema of Swine

Soon after this disease was declared eradicated by the United States Department of Agriculture on October 22, 1959 (1), work with this agent was discontinued at the Beltsville laboratory and samples of all of the types of virus and antisera were transferred to the Plum Island Animal Disease Laboratory which now has responsibility for laboratory assistance in exotic diseases diagnosis. At Plum Island, limited studies were conducted on
complement fixation in the course of cataloguing the materials received from Beltsville. Diagnosis by complement fixation, using serum from infected or recovered swine, was studied and the techniques of the test have been improved. These studies have been reported in a paper presented at this meeting. Briefly, improvements were achieved by partial purification and concentration of the antigen, using ultracentrifugation. Research is continuing on the pathogenesis and genetics of the virus at the University of California at Berkeley under the direction of Dr. S. H. Madin. A comprehensive report on the pathogenesis of vesicular exanthema of swine should be forthcoming from this laboratory in the near future.

**Vesicular Stomatitis**

A long-term study on the changes in tongues of cattle infected with vesicular stomatitis has been reported (2). The initial effects of this virus upon the bovine lingual mucosa was the development of blanched, slightly elevated lesions characterized microscopically by intercellular edema, necrosis of epithelial cells and inflammatory cellular infiltration. Less than 30 percent of the total initial lesions on the tongues of 20 steers inoculated with two strains of the virus developed grossly evident vesicles, including separation of the diseased mucosa from the underlying tissue. These studies agree with clinical findings in recent field outbreaks of the disease in cattle.

**Foot-and-Mouth Disease**

There seems to be a growing tendency towards use of virus propagated on tissue culture cells for vaccine production. Studies on the comparative potency of vaccine produced from this source with that produced from virus from tongue epithelium or explants of infected epithelium (Frenkel technique) are underway.

Vaccines of chicken embryo origin are also undergoing trials in Israel and Africa and perhaps in other countries.

Newer methods in the complement-fixation test now permit differentiation of subtypes where formerly it was necessary to use cattle to determine this. The methods are obviously less expensive (3) (4).

Among other tests, the agar-gel precipitin test is now being used to differentiate among the subtypes of foot-and-mouth disease virus.

It has been reported by Pirbright that intramuscular inoculation of four-eight day old mice will detect minimal quantities of virus (6). Additional evidence has been developed that foot-and-mouth disease virus is circulated widely throughout the body and may be found in parts other than those areas where it produces lesions. Pressure appears to play a significant role in the areas of predilection of the body where lesions are observed (7).

The type and subtype specificity of the interactions between fractions of the virus system of foot-and-mouth disease and its antibody were investigated by complement-fixation methods (8). The 25 mu infective component was shown to combine homotypically with antibody (9).
Preliminary studies have been reported on the use of chemicals other than formaldehyde for inactivation of virus in foot-and-mouth disease vaccines. As Beta-Propiolactone (10), acetylthyleneimine (11), and ethylene oxide (12) are among the new chemicals that are being studied for this purpose.

It has been shown that initial post-partum milk from a first-calf heifer infected with foot-and-mouth disease contained specific neutralizing antibody at a higher level than milk or serum from the same animal at seven days post-parturition. At five days post-parturition, the titers of serum and colostrum were comparable. Complement-fixing antibody was demonstrated in post-partum whey for the first 10 days after parturition but not afterwards. Foot-and-mouth disease virus was not recovered from the meconium, spleen, pancreas, heart muscle or placental tissue of the calves from cows or first-calf heifers infected late in parturition. The clinical signs of foot-and-mouth disease were apparently more severe in pregnant heifers than has been noted in steers or nonpregnant heifers (13).

In studies in mice, it has been shown that pregnant mice and mother mice nursing young were more susceptible to FMDV produced in cultures of bovine kidney cells than were nonpregnant adult mice. Removal of the young one to two days before inoculation resulted in a decrease in the susceptibility of the mother mice. Regardless of the amount of virus inoculated, a maximum of about 40-70 percent of the mothers were susceptible (14). In further studies with mice, it has been shown that humoral immunity was transferred in utero and through milk from immune mother mice to their offspring or suckling mice (15).

In studies underway at the Federal Research Institute, Tubigen, West Germany, FMDV has been serially passaged as many as 500 times in cultures of calf, pig and mouse kidney cells. During this period, the virus changed considerably in virulence for cattle, pigs and baby mice, as well as in cytopathogenicity in various tissue cultures. The virus passaged in pig-kidney cultures lost its virulence as well as its immunizing capacity. Virus passaged in pig-kidney cultures was concluded to be less promising as an immunizing agent than virus passaged in calf and mouse kidney cultures. Type specificity was maintained during the 500 passages in the three different culture media (16). These studies are continuing.

Virus produced in tissue cultures has been found useful in complement-fixation tests and is relatively inexpensive (17). Titers as high as $10^8$ plaque forming units (PFU) per ml. have been produced. This virus promises to be useful in the preparation of vaccines (18).

The studies on the persistence of FMDV in salt-cured meat reported last year (19) have been continued at Plum Island. These studies have shown that FMD, is present in the naemal nodes as well as in lymph nodes in meat from infected carcasses which was subjected to commercial curing procedures (20).
VESICULAR DISEASES

REFERENCES


EVALUATION OF VACCINES FOR VIRUS DIARRHEA OF CATTLE

CHARLES J. YORK*, S. F. ROSNER† and G. J. MACLEAN

Although the disease in cattle known as virus diarrhea was described by Olafson and Rickard (1) in 1946, for a number of years this condition was observed only in widely separated areas such as New York, Indiana (2), and California (3). Another illness called mucosal disease, subsequently described by Ramsey (4), had clinical signs similar in many respects to those originally identified for virus diarrhea, but thought to be caused by a different etiological agent. At first, data indicated that the Indiana variety of virus diarrhea was caused by a different virus than that of the New York type (5). More recently, however, cross-protection tests in cattle have indicated that at least the existing strains of the Indiana virus are identical to those in New York (6).

A number of viruses have recently been isolated from cattle having either virus diarrhea or mucosal disease by investigators using tissue culture procedures, but their importance as causes of these respective conditions has not been clearly determined. Gillespie (7), in 1959, isolated a cytopathogenic virus which, by cross-protection and serological tests, was shown to be identical to the original New York virus diarrhea isolate. Using this virus as a prototype, Gillespie (8) and Kniazeff (9) have compared several viruses isolated from either virus diarrhea or mucosal disease-like illness from different areas of the country, and have shown that apparently most of the viruses isolated to date are antigenically related. This indicates that the virus diarrhea-mucosal disease syndrome is widespread. Furthermore, with the availability of a serological test in tissue culture, it has been possible to determine that there is apparently a high incidence of infection among cattle in the field, with a number of outbreaks of illness identified both by serological tests and virus isolations (10).

Increased recognition of the importance of this disease indicates that a vaccine to prevent it would be desirable. This paper describes studies with two vaccines for this purpose, evaluated in the laboratory as well as under field conditions.

Development of Vaccines. Although details of the development and preparation of virus diarrhea vaccines can be found elsewhere (11, 12, 13), a brief description of the types used in this study is desirable. One consisted of the New York I strain adapted to and modified in virulence in rabbits, and prepared as a modified live virus vaccine using a suspension of infected rabbit tissue. The second vaccine contained the C24V strain of virus diarrhea virus prepared in bovine kidney tissue culture as a modified live virus vaccine.

† Field Consultant, Allied Laboratories, Inc., Omaha, Nebraska.
**Evaluation of Vaccines Under Laboratory Conditions.** Rabbit-adapted or tissue culture vaccine in amounts varying from 0.5 to two ml. was inoculated intramuscularly or subcutaneously into cattle. Ages of the cattle ranged from six months to two years, with most of the common breeds represented. Eight were inoculated with rabbit-adapted virus, nine with tissue culture virus, and 11 were retained as nonvaccinated controls. Temperatures and clinical observations were noted daily for three to four weeks following vaccination. The cattle were then challenged intravenously with two ml. of virulent virus. As may be seen by the results in Table I, there were no signs of illness in experimentally inoculated cattle following injection of rabbit-adapted virus, with the exception of a mild one-day fever in one animal. Leukocyte counts also remained normal. All 19 vaccinated animals resisted challenge which produced typical signs of illness in 10 of 11 uninoculated control cattle.

**Evaluation of Vaccines in Field Cattle.** Studies were undertaken in beef cattle under feed-lot conditions, and in dairy herds, using either rabbit-adapted or tissue culture vaccine. Approximately half of the animals in each group were inoculated, and the remainder held as unvaccinated controls. Serum samples were taken from about 10 percent of both vaccinated and control animals at the time of vaccination, and again two to four weeks later. Serological tests were made on these serum samples to determine the relative immunological status of the cattle at the time of vaccination. The number of cattle given each vaccine is shown in Table II.

### TABLE I

**Vaccination of Experimental Cattle with Virus Diarrhea Vaccines**

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<thead>
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<th>Number of Cattle</th>
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<th>Vaccine Response</th>
<th>Challenge Response</th>
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<tr>
<td>8</td>
<td>Rabbit-adapted</td>
<td>1*/8†</td>
<td>0/8</td>
</tr>
<tr>
<td>9</td>
<td>Tissue culture</td>
<td>0/9.</td>
<td>0/9.</td>
</tr>
<tr>
<td>11</td>
<td>Controls</td>
<td></td>
<td>10/11</td>
</tr>
</tbody>
</table>

* Mild, transient 1-day fever.
† Numerator = number reacting; denominator = number tested.

### TABLE II

**Field Vaccination for Virus Diarrhea**

<table>
<thead>
<tr>
<th>Number of Herds</th>
<th>Number of Vac. Cattle</th>
<th>Number of Controls</th>
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<tr>
<td>13*</td>
<td>1,375</td>
<td>1,130</td>
<td>2,505</td>
</tr>
<tr>
<td>9†</td>
<td>945</td>
<td>493</td>
<td>1,438</td>
</tr>
</tbody>
</table>

* Rabbit-adapted modified live virus.
† Bovine kidney tissue culture modified live virus.
In order to evaluate the success of the vaccines under field exposure conditions, however, the only groups which can be considered are those in which the controls developed symptoms of virus diarrhea. At the time of this report, the controls had sickened in only two groups consisting of 377 feeder lot cattle. Of 112 vaccinated animals, nine showed signs of virus diarrhea, while 67 of 265 controls became ill. This data, analyzed by the Chi 2 test, resulted in a P value of <0.01, indicating that the difference in disease rate between vaccinated and control groups of cattle is highly significant.

Serological Studies. Due to the difficulty of determining precisely the resistance to a field challenge of virus diarrhea that may develop after vaccination, it was felt that serological studies would provide more definitive information regarding effectiveness of the vaccines. Accordingly, serum neutralization tests were conducted with serums of cattle vaccinated with rabbit-adapted or tissue culture virus, and the antibody titers obtained were compared with those of cattle recovered from virulent virus infection. Each of the three groups included experimentally inoculated laboratory cattle and field test cattle. The results are summarized in Table III. As will be noted, no significant difference in serological titers was found for 29 cattle recovered from virulent virus infection, 82 inoculated with rabbit-adapted vaccine, and 65 inoculated with tissue culture vaccine. All animals with negative serology at the time of vaccination developed positive antibody titers two to four weeks post-inoculation, with the exception of one animal given tissue culture vaccine. Titers ranged from 1:20 to as high as 1:10,000 per 0.1 ml. of serum.

Discussion and Summary. Studies on the use of two vaccines for virus diarrhea have been described, one consisting of a rabbit-adapted modified live virus, and the other, a bovine kidney tissue culture modified live virus. Experimentally inoculated cattle successfully withstood challenge with virulent virus diarrhea virus which sickened nonvaccinated controls. Further, in a group of test animals in the field where natural challenge occurred, there was a significant difference in the number of control animals showing signs of illness, as compared to the vaccinates. In fact, the possibility of this difference in disease rate between these two groups occurring by chance is statistically less than 1 to 100.

The serological studies are of even more importance in providing a sound basis for evaluating the vaccines. For a number of diseases, epidemiological evidence and actual experimental challenge have demonstrated that antibodies, when present, denote resistance to specific infectious viruses (14, 15, 16). A recent study by Robson et al. (17) has shown that antibodies determined by neutralization tests are indicators of immunity to virus diarrhea. The results in Table III demonstrate that both vaccines are effective in producing
immunity since vaccinated cattle readily develop antibody titers which are in the same range as those resulting from infection with virulent virus. Further, using the sequential acceptance plan outlined by Robson (18) and the formula

\[ n - x + 1.3448 - \sqrt{\frac{x(n-x)}{n+2.6896}}} \]

where \( n \) is the number of susceptible animals tested, and \( x \) is the number of failures, the lower limit of efficacy (at a 95 percent confidence level) of the rabbit-origin vaccine, based on 81 animals, is at least 95 percent, and of the tissue culture vaccine, based on 65 animals, is at least 93 percent. Therefore, either of these vaccines could be used by the livestock industry to carry out an adequate prophylactic program for prevention of losses caused by virus diarrhea infection.

REFERENCES

VACCINES FOR VIRUS DIARRHEA OF CATTLE


ANTIGENIC RELATIONSHIPS IN THE BOVINE VIRAL DIARRHEA-MUCOSAL DISEASE COMPLEX

A. J. KNIAZEFF and W. R. PRITCHARD

Gainesville, Florida

For many years research on bovine viral diarrhea was impeded by the fact that all the isolated agents were noncytopathic in tissue culture. The isolation by Gillespie et al. (4) of a cytopathic viral diarrhea virus, strain Oregon C24V, made possible the application of numerous virological test procedures employing tissue culture to the exploration of this disease.

The present study† was designed to utilize the advantages of the new strain in the investigation of antigenic relationships in the viral diarrhea mucosal disease complex. An additional aim was to make immunological comparisons between the agents of this complex and several unrelated animal disease viruses. The results are based on virus-serum neutralizations conducted in tissue culture.

MATERIALS AND METHODS

Virus. Bovine viral diarrhea virus (VDV), strain Oregon C24V was kindly provided by Dr. J. H. Gillespie. It was propagated in primary bovine embryonal tissue cultures maintained on a lactalbumin hydrolysate-Hank's solution medium (9).

Virus was harvested after incubation of infected cultures for 72 hours at 36°C., by freezing the cultures once at −65°C. and then thawing at 10°C. Cellular debris was sedimented by centrifugation and the clarified infective tissue culture fluid was stored in sealed vials at −65°C. The average plaque forming unit (PFU) titer of this material was $2 \times 10^6$ per 1.0 ml.

Antiserums tested. In most instances antiserums used in this study came from experimental animals exposed to known viral isolates.‡ Only those antiserums were titrated which had paired preinoculation samples, except in the few instances noted in the following sections.

* Department of Veterinary Science, University of Florida. Supported in part by a grant (E-2268) from the National Institute of Allergy and Infectious Diseases, Public Health Service.

† Preliminary results of this study were presented at the Conference of Animal Disease Research Workers in the southern states, April 1, 1960, at Athens, Georgia.

‡ Some serums used in this study were kindly supplied by the following: R. D. Barner (Michigan State University), N. H. Casselberry (Cutter Laboratories), J. H. Gillespie (Cornell University), G. S. Harshfield (South Dakota State College), R. A. Huck (Ministry of Agriculture, Weybridge, England), D. McKercher (University of California, Davis), F. K. Ramsey (Iowa State College), I. A. Schipper (North Dakota Agricultural College), C. York (Pitman-Moore Co., Indianapolis), and G. Young (University of Nebraska).
Antiserums tested included the following:

Calf antiserums to VDV strain New York (1):
from Florida—four samples;
from Indiana—five samples;
from New York—five samples.

Calf antiserums to VDV, strain not identified:
from Nebraska—one sample.

Calf antiserums to mucosal disease (MDV) agents:
MDV-Indiana (10)—five samples;
MDV-Iowa (14)—eight samples;
MDV-North Dakota (15)—five samples;
MDV-England (6)—four samples.

In addition, antiserums were titrated from animals infected with the following diseases: bluetongue of sheep (two samples), hog cholera (10 samples), infectious bovine rhinotracheitis (two samples), bovine infectious ulcerative stomatitis (four samples), winter dysentery (three samples), malignant catarrhal fever (three samples) and sporadic bovine encephalitis (one sample). Also included were three convalescent serum samples from field cases of bovine mycotic stomatitis (13).

Virus plaque assay. Quantitation of the infective virus in control dilutions and in virus-serum neutralization mixtures was carried out in a plaque assay system characterized by Kniazeff and Walker (8). Primary bovine testicular cell cultures used in this system were propagated in three ounce prescription bottles on a lactalbumin hydrolysate-Hank’s solution medium (9).

Virus-serum neutralization procedures. All serums were inactivated at 56 C. for one half hour prior to use in neutralization tests.

Previously titrated virus which had been stored at —65 C. was thawed at 4 C. and diluted in Hank’s solution to give approximately 350 PFU per 1.0 ml. Aliquots of the diluted virus were then mixed with test serums to give the following final dilutions of serum in virus: for preinoculation serums, 1:10; for convalescent (or post-inoculation) serums, 1:10, 1:100, and 1:1,000. All dilutions were carried out at 4 C. The virus-serum mixtures, as well as proportionately diluted virus controls, were incubated at 25 C. for one hour, following which 0.2 ml. of each sample was inoculated into each of two to four tissue culture bottles. The virus was then allowed to adsorb for one hour at 25 C. The adsorption fluid was removed and a nutrient overlay applied. Plaques were counted after the bottles had been incubated for approximately 90 hours at 36 C.

The specific neutralizing capacities of the antiserums are expressed in terms of percent of plaque inhibition.
Neutralization of Strain Oregon C24V by Bovine Viral Diarrhea Antiserums

The bovine viral diarrhea antiserums from Florida, Indiana, and New York came from experimental calves exposed to VDV strain New York. They were collected 14 to 30 days post infection. All the antiserums showed high and approximately equal neutralizing capacities against strain Oregon C24V. The corresponding preinoculation serum samples were negative in all instances. The Nebraska VDV antiserum also came from an experimental calf, but the virus strain used for animal exposure was not identified. This antiserum neutralized 80 percent PFU at a dilution of 1:100. However, in this case the preinoculation sample neutralized 30 percent PFU at a dilution of 1:10.

The results of these neutralizations are summarized in Table 1.*

<table>
<thead>
<tr>
<th>Antiserum Source</th>
<th>Antiserum Dilution 1/10</th>
<th>Antiserum Dilution 1/100</th>
<th>Antiserum Dilution 1/1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>100</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Indiana</td>
<td>100</td>
<td>92</td>
<td>20</td>
</tr>
<tr>
<td>Nebraska</td>
<td>100</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>New York</td>
<td>100</td>
<td>100</td>
<td>75</td>
</tr>
</tbody>
</table>

* Expressed in percent of plaque units neutralized.

Neutralization of Strain Oregon C24V by Mucosal Disease Antiserums

The antiserums in these titrations came from calves used in studies on four separate mucosal disease virus isolates, from Indiana, Iowa, North Dakota, and England. All neutralized strain Oregon C24V. They averaged 75 percent or higher PFU neutralized at 1:1,000 antiserum dilution. The variation among the neutralizing capacities of the individual samples was less than one log of percent PFU neutralized. Comparison of the average neutralizing capacities of mucosal disease antiserums and VDV strain New York antiserums showed close similarity. Preinoculation samples in this experiment were all negative except for two from Iowa and two from North Dakota which neutralized less than 50 percent PFU in a dilution of 1:10. The antiserums corresponding to these preinoculation samples were not included in the study.

The results of these neutralizations are summarized in Table 2.*

* In this experiment and all following ones, where there were several antiserum samples in a group, their titration results were averaged (i.e., results on the four samples of calf antiserum to VDV strain New York from Florida were averaged, etc.). No group showed significantly divergent results among its samples.
TABLE 2

Neutralization of VDV-C24V With Mucosal Disease Antiserums*

<table>
<thead>
<tr>
<th>Antiserum Source</th>
<th>Antiserum Dilution 1/10</th>
<th>Antiserum Dilution 1/100</th>
<th>Antiserum Dilution 1/1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indiana</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Iowa</td>
<td>100</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>North Dakota</td>
<td>100</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>England</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>

* Expressed in percent of plaque units neutralized.
† Experimental calf antiserums against different mucosal disease isolates.

Results of Neutralization Tests Using Strain Oregon C24V and Selected Disease Antiserums

The diseases considered in this part of the study were: bluetongue of sheep, hog cholera, infectious bovine rhinotracheitis, bovine infectious ulcerative stomatitis, mycotic stomatitis, sporadic bovine encephalitis, winter dysentery, and malignant catarrhal fever.

Neutralization tests using VDV strain Oregon C24V and antiserums to the above diseases were negative except for one malignant catarrhal fever antiserum which neutralized 100 percent PFU at a dilution of 1:1,000. The latter was from an animal which had been inoculated repeatedly with blood and lymph node fluid from acute cases of the disease. A preinoculation serum sample was not available for this antiserum.

The results of these titrations are summarized in Table 3.

TABLE 3

Neutralization Tests With VDV-C24V and Selected Antiserums*

<table>
<thead>
<tr>
<th>Antiserum Types</th>
<th>Antiserum Dilution 1/10</th>
<th>Antiserum Dilution 1/1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluetongue (sheep)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hog Cholera</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infectious Bovine Rhinotracheitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infectious Ulcerative Stomatitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mycotic Stomatitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant Catarrhal Fever (acute)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant Catarrhal Fever (&quot;hyperimmune&quot;)†</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sporadic Bovine Encephalitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Winter Dysentery</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Expressed in percent of plaque units neutralized.
† No preinoculation serum.
DISCUSSION

The existence of antigenic relationship between viral diarrhea virus strain Oregon C24V and strain New York has been demonstrated by Gillespie et al. (4) and by Kniazeff and McClain (7). In the latter study the two strains were compared by cross-neutralization in tissue culture, using a technique based on the virus interference phenomenon. Results of these cross-titrations showed that the two strains are probably antigenically identical.

Neutralizations of strain Oregon C24V with VDV strain New York antiseraums (summarized in Table 1) showed high, specific, and closely equivalent neutralizing capacities for antiseraums obtained from three different sources, indicating that calf antiseraums can be used with a considerable degree of reliability in the antigenic comparison of these agents.

Antiserums for several animal diseases having some similarity in their pathology or pathogenesis to the virus diarrhea-mucosal disease complex were titrated. The results were negative in all cases but one (see Table 3). This bespeaks the high specificity of the neutralization system used.

On the basis of the above considerations, it appears justifiable to use VDV strain Oregon C24V in a neutralization system to investigate antigenic relationships in the viral diarrhea-mucosal disease complex.

Titration results on mucosal disease antiseraums representative of the viral isolates from Indiana, Iowa, North Dakota, and England (summarized in Table 2) present conclusive evidence of antigenic relationship between these isolates and VDV strain Oregon C24V. The consistently high, specific (judging by negative preinoculation samples), and practically equivalent neutralizing capacities of these antiseraums substantiate this conclusion.

In view of the unquestionably close antigenic relationship between VDV strains Oregon C24V and New York, it may be assumed that an antigenic relationship also exists between the latter strain and the four mucosal disease isolates. Further, it may be stated that such a relationship extends to other viral diarrhea-mucosal disease isolates previously shown to be antigenically similar to strain New York. These would include such strains as Indiana 46 (11) (3), California (16), and several recently recovered agents from Nebraska and New York (5).

The over-all immunological evidence so far obtained leads to the conclusion that the viral diarrhea-mucosal disease agents here considered are members of an antigenically related group. This relationship, in conjunction with the highly similar pathogenicities exhibited, provides positive grounds for considering these isolates as etiologic agents of a single disease syndrome. Their differences in epizootiological and pathological characteristics do not invalidate this conclusion. On the other hand, the present immunological evidence should not be taken to imply that all of the agents of this syndrome must be antigenically related. In fact, data contradicting this assumption already exist. Thus, Pritchard et al. (10) reported the isolation of a viral diarrhea agent (strain Indiana 143) distinct from strain New York. In addition, more recent studies by York (17) show that a certain percent of
ANTIGENIC RELATIONSHIPS—DISEASE COMPLEX

Antisera collected in field outbreaks of viral diarrhea do not neutralize strain Oregon C24V. In order to clarify this aspect of the viral diarrhea problem, more emphasis must be placed on the isolation and typing of new agents.

Unfortunately, the experimental design of this study as well as that of Gillespie et al. (5) do not permit exact evaluation of antigenic interrelationship between strains. Such evaluations, which are absolutely essential for the segregation of strains into antigenic types, can be accomplished only through quantitative cross-titration tests. For complete characterization of the isolates, additional studies must be undertaken to define their biophysical, chemical, and pathogenic characteristics in cell cultures and in animals. Such studies are now in progress.

The neutralization results with antisera listed in Table 3 did not indicate any relationship between strain Oregon C24V and the following: bluetongue of sheep, hog cholera, infectious bovine rhinotracheitis, infectious ulcerative stomatitis, mycotic stomatitis, sporadic bovine encephalitis, or winter dysentery. The tests on hog cholera were conducted with post-vaccinal and post-challenge swine antisera. Negative neutralization results indicate that the reported antigenic relationship between hog cholera and mucosal disease (2) most likely is dependent upon antigens not participating in the virus-serum neutralization phenomenon.

In the absence of a preinoculation serum, the presence of VDV neutralizing antibody in a malignant catarrhal fever antiserum cannot be considered indicative of antigenic relationship. Properly controlled experiments should be conducted to test this point.

SUMMARY

Neutralization of a cytopathic bovine viral diarrhea virus, strain Oregon C24V with antisera to several bovine viral diarrhea-mucosal disease agents revealed that these agents form an immunological related group.

Neutralization tests did not show any relationship between viral diarrhea virus, strain Oregon C24V, and the following: bluetongue of sheep, hog cholera, infectious bovine rhinotracheitis, infectious ulcerative stomatitis, mycotic stomatitis, sporadic bovine encephalitis, or winter dysentery.

REFERENCES


5. Gillespie, J. H., Coccins, L., Thompson, J., and Baker, J. A.: Comparison by Neutralization Tests of Strains of Virus Isolated from Virus Diarrhea and Mucosal Disease. (To be published.)


During the past few years, a great many “new” viruses have been isolated from most species of domestic animals. The bovine, porcine and feline species appear to be hosts to the largest variety of virus infections at present, but this may be only the result of current methods of approach. In studying the virus etiology of any disease, the first step after making an isolation should be to determine whether the new isolate is identical to, or different from, agents already obtained by the investigator or by another group. All too frequently when a virus is isolated, it is studied from various approaches without too much consideration being given to its possible relationship to agents isolated elsewhere from the same species of animal, regardless of the source of the specimen. Even when an attempt is made to identify a new virus or group of viruses, the methods employed vary widely, and comparisons between laboratories are difficult.

In order to correlate research, recommend standard methods, and suggest a means of classifying animal viruses, a Committee on Virus Research was formed last year by the United States Livestock Sanitary Association. Its purpose, in part, is to work out a satisfactory procedure for exchanging viruses and sera between laboratories for identification purposes; to formulate an acceptable, detailed, standardized method of virus identification by cooperating investigators; and to devise some means whereby an effective classification may be utilized to avoid confusion in the rapidly expanding field of etiology of virus infections of domestic animals. The Committee held several meetings, attended by a majority of the men actively engaged in veterinary virus research. The following is a summary of the discussions and course of action recommended.

PART I

1. In order to identify viruses already isolated in laboratories throughout the country, a means of serological typing is necessary. A proposed standard method for serological identification of viruses has been drawn up in order to facilitate comparison of results obtained by different groups. This draft is to
be reviewed by the committee members before the final form is prepared. The target date for completing this is November 20, 1960.

2. In order to expedite the use of this standard outline by the various laboratories, a coordinator for each species of domestic animal will be selected from the committee membership. The species to be supervised in this manner are bovine, porcine, feline, canine, and poultry.

3. A liaison officer will be chosen to assist the species coordinators. His function will be to keep in touch with the activities of each group and to provide advice and assistance, both scientific and fund-raising, where needed.

4. The species coordinators will be responsible to the Committee on Virus Research.

PART II

Although antigenic identification of viruses is the first step in clearing up the confusion that exists in the field, there remains the problem of naming or classifying the agents by some standard method. In order to solve this phase, the following was proposed by the Committee:

1. Nomenclature for viral agents in the field of veterinary medicine should follow as closely as possible the system adopted by the groups interested in viral agents of primates.

2. A group of committee members active in the field of virology will be appointed to prepare a form requesting information on the currently known biological, physical and chemical characteristics of various viruses which would aid in their subsequent classification.

3. The group responsible for drafting this form will meet to initiate this project early in 1961 at the National Animal Disease Laboratory in Ames, Iowa. It is further suggested that the individuals concerned plan to spend a minimum of one week on this task, after preparing themselves as thoroughly as possible.

4. This form, when prepared, should be sent to the men working in cooperating laboratories to obtain the necessary information on various viruses. When the completed forms have been received, the chairman will reconvene the original group, and other individuals if necessary to establish a system of classification compatible with current information, which will also provide a degree of flexibility for future revisions.

The results of this work should be published in pertinent journals as a joint effort of the cooperating laboratories. Abstracts of such papers will also logically be included in future committee reports.

As part of the future program, investigators who identify a virus or group of viruses should be willing to supply a pool of virus and antiserum for use
as reference in future research. It is recommended that the National Animal Disease Laboratory at Ames act as the repository for these viruses and sera, and as a central agency for their use.

It is recommended that the Executive Committee of the United States Livestock Sanitary Association give permanent standing to the Committee on Virus Research for the next few years at least. In order to provide continuity, it is also suggested that, if possible, the chairman serve for at least two consecutive years.

It is also considered desirable, if there is no objection from the Executive Committee, to invite a scientist from Canada and from Mexico to meet with the Committee on Virus Research in order to keep these countries abreast of its activities, and to pave the way for exchange of information in the future.

The following committee assignments were made:

- Dr. R. C. Reisinger, Coordinator for cattle viruses
- Dr. W. P. Switzer, Coordinator for swine viruses
- Dr. R. A. Bankowski, Coordinator for poultry viruses
- Dr. R. A. Crandell, Coordinator for cat viruses
- Dr. J. H. Gillespie, Coordinator for dog viruses
- Dr. W. R. Hinshaw, Liaison Officer
- Dr. S. H. Madin, Chairman, Subcommittee on Virus Classification.
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 disbursments of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.

ARTICLE VI—PROGRAM COMMITTEE

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

ARTICLE VII—DUTIES OF OFFICERS

1. President: It shall be the duty of the president to preside at all meetings of this Association; to appoint all committees excepting the Executive and Officer 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.

**Article V—Dues**

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

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

The dues for official memberships shall be fifty dollars ($50.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
3rd ANNUAL MEETING
CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS
October 17-18, 1960
CHARLESTON, WEST VIRGINIA
R. A. BANKOWSKI, President, Davis, California
E. P. POPE, Secretary, Madison, Wisconsin

2. Effects of Ionizing Radiations and Diagnostic Problems, A. C. Anderson.
9. Laboratory Confirmation of Encephalomyelitis in Horses, Robert J. Byrne.
10. Diagnosis of Infectious Synovitis, N. O. Olson.
STANDARD NOMENCLATURE OF ANIMAL DISEASES AND OPERATIONS

D. K. SORENSEN, College of Veterinary Medicine

University of Minnesota

The problem of nomenclature of diseases and operations is one of tremendous scope and magnitude. It has plagued physicians, veterinarians and other branches of medical and veterinary science for years. We have many groups working on nomenclature at present and progress is slow at best. Virologists and bacteriologists cannot agree on what to name new micro-organisms or whether to change the names of old ones. Physicians use several synonyms for the same disease as do veterinarians. Newly recognized diseases present problems as to what to name them and it frequently takes years for a satisfactory terminology to evolve. My objective in this paper is to explain the present status of nomenclature of animal diseases in veterinary medicine today.

There are essentially three aspects to the problem of nomenclature: The classification of diseases and operations, the terminology or nomenclature and the naming of new diseases. Each category presents special problems and at present are being handled in a variety of ways. In general, in the past there has been no concerted effort to standardize the naming of new diseases or the terminology of existing diseases. There has been an effort made to classify animal diseases and operations. To better explain the present Standard Nomenclature of Animal Diseases and Operations, a historical review as to its origin will be presented. This system of classifying disease was developed by the medical profession. Work on this nomenclature was initiated by invitation of the New York Academy of Medicine in 1928. A National Conference on Nomenclature of Disease was formed with a membership representing most of the leading medical and public health organizations in the country. The steering committee of this conference prepared the plan for a dual topographical, etiological classification in accordance with the library coding system. This basic plan was adopted at the second national conference in 1930. The first printing was then published in 1932 and a second edition in 1935. Since a nomenclature of this kind must be kept abreast of the progress of medicine, the responsibility for its periodic revision was taken over by the American Medical Association in 1937. At present an editorial advisory board of the American Medical Association is responsible for its revision. They have 22 committees to aid them in revising the book.

We have not yet explained the standard nomenclature. It attempts to include every disease which is clinically recognizable. It has been designed primarily for use by clinicians, as the clinical diagnosis is a most important source of information of prevalence and distribution of disease.
The method of classification is based on two elements: The portion of the body concerned (topographic) and the cause of the disease (etiologic). These two elements are designated by code numbers separated from each other by a hyphen. The first three digits describe the topographic site; the last three, following the hyphen, describe the etiologic agent. Combined they form a complete diagnostic code number.

**TOPOGRAPHIC CLASSIFICATION**

The main topographic divisions are:

0—Body as a whole including the psyche and the body generally) not a particular system exclusively.

1—Integumentary System (including subcutaneous areolar tissue, mucous membranes of orifices and the mammary gland).

2—Musculoskeletal System

3—Respiratory System

4—Cardiovascular System

5—Hemic and Lymphatic System

6—Digestive System

7—Urogenital System

8—Endocrine System

9—Nervous System

X—Organs of Special Sense

These major groups are further divided in order to specify a definite organ or part of an organ. Thus for example, the digestive system is designated by six. The fourth organ listed in the system being stomach, the digits for the stomach are 64. The pylorus which according to arrangement is the fifth structure under stomach therefore receives the code number 645. Thus if a lesion involves the whole alimentary tract, it will receive the topographic classification 600—; if the disease involves all of the stomach, it will receive the number 640—; and if it can be positively identified as involving the pylorus, it receives the number 645—.

**ETIOLOGIC CLASSIFICATION**

A similar system of numbering the causes of disease constitutes the second element of the classification. Thirteen major classifications of etiology are included.

—0 Diseases due to prenatal influence

—1 Diseases due to lower plant or animal parasite

—2 Diseases due to a higher plant or animal parasite

—3 Diseases due to intoxication

—4 Diseases due to trauma or physical agent

—50 Diseases secondary to circulatory disturbance

—55 Diseases secondary to disturbance of innervation or of psychic control
-6 Diseases due to or consisting of static mechanical abnormality (obstruction, calculus, displacement or gross change in form) due to unknown cause
-7 Diseases due to disorder of metabolism, growth or nutrition
-8 New growths
-9 Diseases due to unknown or uncertain cause with the structural reaction (degenerative, infiltrative, inflammatory, proliferative, sclerotic or reparative) manifest; hereditary and familial diseases of this nature
-X Diseases due to unknown or uncertain cause with the functional reaction alone manifest; hereditary and familial diseases of this nature
-Y Diseases of undetermined cause.

As in the topographic classification, these major groups are further subdivided to specify particular etiologic agents. For example, a causative agent identified as a poison, but with exact nature undetermined or unspecified, receives the number —300. If identified as a metallic poison, but with the exact metal undetermined, it will receive the number —311, and if identified as mercury it receives the number —3111, thus indicating the specific etiologic agent.

To further point out how the system works an example of a disease for each etiologic classification of the Respiratory System is cited.

**Respiratory System**

**Diseases of the Lung**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>360-019</td>
<td>Atelectasis, congenital</td>
</tr>
<tr>
<td>360-16042</td>
<td>Swine influenza</td>
</tr>
<tr>
<td>360-226</td>
<td>Aspergillosis of lung</td>
</tr>
<tr>
<td>360-300</td>
<td>Edema of lung due to poisoning</td>
</tr>
<tr>
<td>360-400.4</td>
<td>Collapse of lung, due to trauma</td>
</tr>
<tr>
<td>360-500.4</td>
<td>Embolism, pulmonary, due to circulatory disturbance</td>
</tr>
<tr>
<td>360-600.6</td>
<td>Emphysema, compensatory</td>
</tr>
<tr>
<td>360-8091A</td>
<td>Adenoma of lung</td>
</tr>
<tr>
<td>362-9X6</td>
<td>Emphysema, pulmonary, due to unknown cause</td>
</tr>
<tr>
<td>360-YX7</td>
<td>Pulmonary hemorrhage</td>
</tr>
</tbody>
</table>
The veterinary profession made its first start towards a uniform system of nomenclature when the American Veterinary Medical Association's Special Committee on Nomenclature of Diseases decided to adapt this Standard Nomenclature of Diseases and Operations developed by the American Medical Association. This Committee then further developed the system by revising the topographical and etiologic classifications to enable it to be used for diseases of animals. In 1955 this special Committee on nomenclature prepared and published the topographic classifications and etiologic categories which was the first important step in developing the standard nomenclature system. They then recommended that the clinicians at the veterinary colleges further develop the system. In 1957 the American Association of Veterinary Clinicians was formed. This group is composed of clinicians of the veterinary colleges and at their second meeting in 1958 assumed responsibility for further developing this system. Dr. Harvey Hoyt was appointed the first chairman of this Committee. Using the topographic and etiologic classification adapted from the standard nomenclature of diseases and operations a rather comprehensive list of medical and surgical diagnoses has been prepared. This standard nomenclature is now in use in most of the veterinary colleges in the country.

We are in the process of completing and preparing a final list to be published and distributed. Before this can be done we have to appoint subcommittees to edit each system to insure we have every known disease and operation included. These subcommittees would also list the preferred terminology or nomenclature of each disease as well as the synonyms. For example:

<table>
<thead>
<tr>
<th>Code Number</th>
<th>Preferred Name</th>
<th>Synonym</th>
</tr>
</thead>
<tbody>
<tr>
<td>010-750</td>
<td>Ketosis, bovine</td>
<td>Acetonemia</td>
</tr>
</tbody>
</table>

It is anticipated that the subcommittees consist of the leading authorities in a particular field wherever possible. So there still remains much to be done on developing the system and putting it into operation.

At present this subcommittee of the American Association of Veterinary Clinicians also functions as a subcommittee of the Council on Education Committee of the American Veterinary Medical Association. The council on education is interested in developing this system for standardized reporting from all the veterinary colleges. To fully implement and develop the system we will need the help and cooperation of all groups in the veterinary profession.

The objectives of the Standard Nomenclature of Animal Diseases and Operations are as follows:

1. Develop a standard system for reporting diseases and operations.
2. Revise and standardize the terminology of diseases.
3. Develop a standard system for the naming of new diseases.
More than six decades have passed since ionizing radiations were discovered; in 1895, Wilhelm Roentgen announced a "new form of energy," and a few months later, radioactivity was reported by Henri Becqueral (1). The biological effects of ionizing radiations were soon recognized, and by the turn of the century their local, systemic, and therapeutic powers were known.

Scientific advances since the discovery of ionizing radiations have placed us in the atomic era, with an ever-increasing opportunity for radiation exposure. Despite current scientific knowledge of this field, it is commonly recognized that actual detection of the radiation source is the only authentic means of diagnosing radiation exposure. Lethal or near-lethal whole-body irradiation may be suspected from the pattern of symptoms and post-mortem lesions, but difficulties arise because of individual and species variations. Lower levels of irradiation usually require a lapse of several months to years before producing a demonstrable effect. Similar problems are encountered with short-lived internally deposited radioisotopes, but long-lived and retained internal emitters may be accurately detected by the informed diagnostician.

This report demonstrates the general features of both whole-body irradiation (X, γ, and nγ rays) and radioisotopes damage in relation to diagnostic problems in mammals. Short-lived (I-131) and long-lived (Sr-90) radioisotopes are compared to illustrate these features. Those interested in a more detailed account of radiation effects are referred to texts dealing with this subject (2, 3, 4, 5, 6).

RADIATION TERMINOLOGY AND FUNDAMENTAL CONCEPTS

Radiation is defined as the sending forth or giving out of rays, and irradiation as subjection or exposure to radiant energy (7). We are concerned with penetrating radiations—invisible waves or particles that are able to penetrate matter. It is well known that every living creature is subjected to small amounts of radiation, because ionizing radiations are a part of our environment. Our problem concerns more concentrated types of radiation, naturally or artificially produced, the most common being roentgen (X-rays), gamma, alpha, beta, and neutron rays. These rays, either electromagnetic waves or particles, lose energy by ionization while passing through matter. Ionization is the dissociation of molecules into positive and negative ions (free radicals), hence the term ionizing radiations. Either directly or indirectly, ionizing radiations produce a biological response by: affecting an enzyme system, denaturing proteins, altering colloids, or changing the viscosity of a solu-

* A. E. C. Projects 4 and 6, School of Veterinary Medicine, University of California, Davis.
EFFECTS OF RADIATIONS AND DIAGNOSTIC PROBLEMS 365

With few exceptions, ionization occurs at the time of irradiation, but a "time-lag" always follows a demonstrable effect. The "time-lag" may vary from minutes to years, depending on the dose received. Radiation dose is commonly expressed in roentgens (r) or rads; the roentgen is an air measurement approximating 93 ergs/gm tissue, and the rad designates 100 ergs/gm tissue. An example of radiation dose and "time-lag" in mammals may be illustrated as follows: 10,000 r = minutes (peracute); 1,000 r = days (acute); 100 r = months to years (long-term); and one r = questionable.

Irradiation is always damaging; any stimulation is actually a response to injury, or impaired vital function (10). A single radiation dose is more efficient in producing an effect than the same total amount given in multiple small doses. Species lethality of acute single exposures is as follows: mouse, rat, guinea pig, hamster, rabbit, and monkey fall into one classification, with a 30-day LD₅₀ between 400 and 800 rads, whereas larger animals (dog, pig, sheep, goat, burro, and probably man) are in another classification, with an LD₅₀ between 200 and 300 rads (11).

FUNDAMENTALS OF IONIZING RADIATIONS

I—Ionizing Radiations:

(a) Radiation  (b) Type of Radiation  (c) Irradiation
(1) electromagnetic  
(2) particle

II—Irradiation Causes Ionization, the Dose or Exposure Is Expressed as Roentgen (r) or Rad.

Example:

Radiant energy + H₂O → H⁺ + OH⁻ or H₂O₂ or HO₂
H₂O₂ + Protein → denatured, etc.

III—Ionization Produces an Effect Which Is Observed After an Elapse of Time.
IV—Dose Versus Biological Effect.

10,000 r — Immediate, peracute
1,000 r — Days, acute
100 r — Weeks, chronic (long-term)
10 r — Years (long-term)
1 r — Questionable

EFFECTS OF IONIZING RADIATIONS

Although effects vary with type and dose of radiation, species, and age of the animal, a characteristic pattern of symptoms and lesions follows irradiation. These are best summarized in terms of: (1) a mid-lethal whole-body exposure of X or gamma rays, and (2) the general characteristics of radioisotope damage.

1. Whole-body X-irradiation (MLD)

A whole-body exposure to X, γ, or neutron rays in the mid-lethal range is generally accidental or experimental, from X-ray machines, cyclotrons or nuclear devices. During and shortly after exposure, the animal may show a nausea that soon subsides. A seven- to 12-day period of well-being follows. A typical example is shown in Figure 1. Dog A received 300 r (LD_{50/30})

![Diagram A: 300 r X-irradiation](image)

![Diagram B: 350 r X-irradiation](image)

**Figure 1.** Symptoms and peripheral blood changes in the beagle typifying acute radiation sickness. (A) 300 r. (B) 350 r. Whole-body exposure 250 Kvp, 30 ma, HVL 2.65 mm. Cu.
but exhibited only partial loss of appetite and moderate diarrhea, with recovery 20 days after exposure. Dog B, in contrast, received 350 r (LD$_{90}$/30) and showed pronounced symptoms, although failure in general condition and evidence of death were not apparent until some two weeks after exposure. During the period of well-being, a distinct subclinical syndrome occurred; lymphopenia was followed by leukopenia and thrombocytopenia.

Although not readily apparent from peripheral blood examination, bone-marrow cellularity greatly decreases within four to six days of irradiation. As illustrated in Figure 2-A, B, bone-marrow cellularity, except for mature and stem cell elements, has been largely replaced by adipose tissue. Reduced mitosis is also apparent in such rapidly dividing tissues as lymph nodes, intestinal tract, and gonads. The sensitivity of dividing cells to radiation has long been recognized, as stated in 1906 in the law of Bergonie and Tribondeau (10).

The early onset and severity of symptoms (emesis, diarrhea, elevated temperature) and subclinical manifestations (leukopenia, thrombocytopenia) following irradiation do not offer a definitive prognosis for either death or survival. An occasional animal exhibiting drastic signs of radiation sickness to the extent of becoming moribund, may recover; the usual result of acute radiation, however, is death 14 to 21 days after exposure.

The dog shown in Figure 3 remained in good general condition until two days before this photograph. During this two-day period, she became progressively more depressed, refused food, developed a profuse bloody diarrhea, and hemorrhaged from the mucous membranes. Her outward appearance (glossy haircoat and good flesh) gave little evidence that death would occur 12 hours later. At autopsy, widespread hemorrhage and varying

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**Figure 2.** Bone-marrow cellularity following a 300 r whole-body X-ray exposure. (A) Normal. (B) 6-days post-irradiation. Midfemur samples at sacrifice. Bouin's hematoxylin and eosin, 100X.
Figure 3. Beagle, 12 hours prior to death following a mid-lethal (300 r) exposure 15 days previously. Gross autopsy findings shown in Figures 4, 5, 6 and 7.

Figure 4. At autopsy, the beagle shown in Figure 3 revealed widespread hemorrhage. (A) Pyers patch. (B) Thoracic cavity organs.
Figure 5. Closer inspection of thoracic cavity organs after removal of ventral lobes of the lung, revealing (A) widespread ecchymotic and (B) confluent hemorrhagic lesions, (C) liver.

Figure 6. Stomach and neighboring viscera showing (A) hemorrhage of stomach wall and (B) mesenteric lymph node. (C) Liver.
Figure 7. Interior (mucosal surface) of stomach shown in Figure 6. (A) Petechial and ecchymotic lesions. (B) Necrotic areas in fundic region.

Figure 8. Coronary vessel from Beagle succumbing to 300 r whole-body X-radiation revealing (A) intravascular polysaccharide, PAS-positive in contrast to (B) erythrocytes being PAS-negative. Alcohol-formal, nitrocellulose, periodic-acid-Schiff stain. 200X.
amounts of necrosis were readily detected, certainly explaining her moribund condition (Figures 4, 5, 6, 7). These figures, enlarged views of thoracic cavity and visceral organs, illustrate the characteristic features of radiation-induced hemorrhage. In general, petechial lesions are circular, becoming larger and irregular confluent hemorrhagic areas. The cause of radiation-induced hemorrhage is believed to be thrombocytopenia, although associated phenomena have been advanced (10).

The findings of emboli in radiation-induced hemorrhagic lesions has been consistent in this laboratory (13), and this substance (Figure 8) could be of interest in radiation diagnosis. Fifty-two dogs succumbing to acute radiation revealed the presence of this intravascular substance. It has characteristic staining features, is strongly PAS-positive (periodic-acid-Schiff) and acidophilic, but negative for lipids and erythrocytic products. It can be identified with ease in the dog and pig, and with difficulty in the guinea pig; other species examined have shown the substance as globules smaller in diameter than erythrocyte, making its identification more difficult (see Table 1) (14).

<table>
<thead>
<tr>
<th>SPECIES DIFFERENCE</th>
</tr>
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<tbody>
<tr>
<td><strong>INTRAVASCULAR POLYSACCHARIDE POST IRRADIATION</strong></td>
</tr>
<tr>
<td>SPECIES</td>
</tr>
<tr>
<td>Pig</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Man</td>
</tr>
<tr>
<td>Guinea Pig</td>
</tr>
<tr>
<td>Monkey (Java)</td>
</tr>
<tr>
<td>Rat, FAC(I) F1</td>
</tr>
<tr>
<td>Hamster</td>
</tr>
<tr>
<td>Mouse, CAF1</td>
</tr>
<tr>
<td>Rabbit</td>
</tr>
</tbody>
</table>

(1) 250 kv, HVL = 1.0 - 2.6 mm Cu, X-rays.
(2) Gross and subgross.
(3) Pollock’s trichrome stain.
Although requiring additional study, the finding of this same intravascular substance in different species and its apparent specificity to radiation damage does offer a possible tool for diagnosis.

Of the two groups (Table I), the hemorrhagic-prone group is more susceptible to radiation, the median lethal dose being less than 400 rads. As shown in Figure 9, massive hemorrhage (A) is the predominant lesion at autopsy after acute radiation exposure. In contrast, the hamster is an example

Figure 9. Guinea pig, 12 days after a 350 r (X-ray) exposure. (A) Widespread hemorrhage.
where a higher (650 r) lethal exposure without massive hemorrhage is characteristic at autopsy.

Survivors of acute radiation damage may live for years before the effects are demonstrable. Except for mice, which become sterile after 50 r whole-body irradiation (15), female and male animals merely exhibit a temporary loss of fertility after 300 r. An extensive study of female dogs given median lethal exposures of X and γ rays revealed that fertility was not impaired until the dogs showed signs of early aging (see Table II) (16). If this holds

TABLE II

| Estrous Interval and Fertility in Beagles After Whole-body X-irradiation |
|-----------------------------|-----------------------------|-----------------------------|
| Number of Dogs              | 0                           | 100 r                       | 300 r                       |
|                             | 29                          | 65                          | 76                          |
| Estrous Interval—           |                             |                             |                             |
| (a) Puberty to 1,500 days  | 216 ± 53.5                  | 222 ± 66.7                  | 220 ± 62.3                  |
| (b) 1,500 to 2,500 days     | 235 ± 85.3                  | 240 ± 85.2                  | 259 ± 179.0                 |
| Number of Breedings         | 61                          | 134                         | 190                         |
| Number of Whelpings         | 50                          | 115                         | 156                         |
| Pups per Litter             | 4.8                         | 4.7                         | 4.8                         |
| Fertility, Percent          | 82.0                        | 85.8                        | 89.7                        |

true for other mammals, the problem of mutation becomes increasingly important since it is generally accepted that 50 rad doubles the rate (17).

The late sequelae following irradiation include: premature graying, accelerated aging, cataracts, neoplasms, and shortened life span. Among domestic animals the more important of these syndromes are probably accelerated aging and neoplasms. The accelerated aging and shortened life span induced by irradiation were formerly thought to be merely a nonspecific reaction to injury, but, as far as is known radiation is a unique stress that is not clearly understood (18).

It is generally accepted that ionizing radiations rank second to none in potency as a single carcinogenic agent (19). In man, the first radiation-induced cancer (skin) was recognized in 1902 (20). Subsequent reports for both man and laboratory animals have clearly demonstrated the carcinogenic action of radiations, but the mechanisms involved are not clearly understood. It is the opinion of most researchers that there is no radiation threshold for tumor induction (21, 22, 23). Many different types of both benign and malignant radiation-induced tumors have been reported, but a new type of neoplasm has not been induced by irradiation. Few reports exist on radiation-induced neoplasia among domestic animals, but accumulated information is sufficient to warrant the conclusion that these species (dog, cat, sheep, goats, cattle, etc.) respond similarly to man and laboratory animals. One case of radiation-induced lymphocytic leukemia was suspected in 56 York-
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shire-Hampshire hogs exposed to gamma radiation (Co-60 source) (24). Our long-term study of X-irradiated dogs recently revealed two cases of ovarian adenocarcinoma (25). At present, these cases can only be assumed to be radiation-induced; two cases have occurred among 132 beagles to 300 r, whereas none were observed among 56 controls or 146 beagles exposed to 100 r. Since spontaneous ovarian adenocarcinomas do occur in the dog (26, 27) the only means whereby these cases can be accurately diagnosed as radiation-induced is by statistical means; this will require several additional years of study. In our experiment, ovarian carcinomas developed after latency periods of 1,550 to 1,820 days from radiation. The number of animals and the time required to produce neoplasms from external irradiation no doubt explain the scarcity of reports on this subject among domestic animals. This is not true with long-lived internal emitters, where the radiation source can be detected.

2. General Characteristics of Radioisotope Damage

Isotopes are atoms with different atomic mass but identical chemical properties; some 300 stable and 800 radioactive isotopes have been identified (1). A radioactive isotope behaves physiologically like its stable daughter, the difference being in the release of radiant energy during decay. Thus, the mode of damage from a radioisotope varies with its physical and physiological properties, since each has a characteristic half-life, type and energy of radiation. These factors are important to both radiation damage and diagnosis. Typical contrasts are illustrated here with radioiodine (I-131) and radiostrontium (Sr-90).

Radioiodine (I-131) has a physical half-life of eight days and emits both beta and gamma rays with an effective decay (physical and physiological) of about six days (28). As reviewed by Cooper (28), radioiodine behaves similarly to natural iodine: upon entering the body, I-131 is soon concentrated in the thyroid gland. If concentration is sufficient, I-131 damages the thyroid gland, largely (90 percent) from beta radiation. The extent of damage varies from partial to complete thyroidectomy, which causes a variety of symptoms that become apparent several weeks to months after I-131 has decayed. An experiment was conducted with 16 beagles, 2½ to three months old, given intravenous doses of 0.5 to 5.0 mc I-131 (29). The eight dogs given doses from 2.5 to 5.0 mc I-131 began to show abnormal development three to four months after treatment. When compared to their controls, dogs injected with I-131 exhibited a "bulldog" appearance; their head, neck, shoulders and gait were characteristic of the bulldog rather than the beagle breed. These symptoms as well as signs of dry haircoat with excessive wrinkling of the skin increased with advancing age. A few days after the photograph was taken (Figure 10), this dog was sacrificed for pathological study, and careful dissection of the thyroid region revealed a unilateral, small (0.25 cm in diam.) hardened mass of tissue. Histological examination revealed atypical and presumably nonfunctional thyroidal tissue (Figure 11). Five of the eight dogs in this experiment showed similar clinical symptoms,
Figure 10. Beagle 25A, 30 months after receiving an intravenous dose (3.2 mc I-131) of radioiodine. Note timid expression, abnormal conformation, and wrinkled skin.

Figure 11. Thyroidal mass found at autopsy of beagle 25A (Figure 10). Note parathyroid and abortive attempt to regenerate thyroid tissue after 3.2 mc I-131. Formol, hem, and eosin, X100.
and autopsy showed partial to complete thyroidectomy without parathyroid
damage. Pathognomonic lesions of radioisotope damage were limited to the
thyroid. Thus, without a previous history of radiation exposure, the etiology
of such cases of hypothyroidism would be difficult to diagnose. Similar
diagnostic problems exist for all short physical and biological half-life
radioisotopes.

Radioisotopes with a long physical and biological half-life are more con-
ductive to radiation damage—and more easily detected. That is especially
true of those that emit energetic radiation during decay. Sr-90 is an excellent
illustration of these features. It behaves biologically like calcium, has a
half-life of 28 years, and decays by beta emission to Yttrium-90, which has
a short half-life (64 days) but emits a more energetic and damaging beta
ray (20). Since the man-made contamination of our environment with Sr-90,
this radioisotope has had widespread interest among both scientist and the
public. This report does not dwell on Sr-90 as a radiation hazard, since we
are more concerned with its mode of damage and detection. Those interested
in the current status of Sr-90 in relation to fallout are referred to the recent
report by Dunham (31).

The ingestion of Sr-90 is followed by skeletal deposition; it is a “bone-
seeker,” and about five percent of the ingested dose becomes fixed in the

Figure 12. Roentgenogram (dog 5S1) revealing osteogenic sarcoma of 4th, 5th, and
6th lumber vertebrae following Sr-90 administration.
About four years ago, we began an experiment in which Sr-90 was fed daily to beagles from birth to 18 months of age (32). A litter of six beagles were fed ad libitum a ration containing about 1.83 uc Sr-90/gm dietary Ca, and two of these dogs (5S1 and 5S2) were maintained for long-term studies. About 20 months after the “spiked” ration was stopped, animal 5S1 developed posterior paralysis, and roentgenograms revealed a small tumor in the fifth lumbar vertebrae. As shown in Figure 12, within eight weeks of recognition, this tumor encompassed about one-half of the lumbar region. The animal was then sacrificed for pathological study, and the tumor was diagnosed.
histologically as an osteogenic sarcoma. No other tumors were found in this
dog, but bone irregularities were readily apparent. A 5-mm slice of the
femur placed on X-ray film produced an autoradiograph (Figure 13-A) that
corresponded to the bone concentration of Sr-90. Variations in the exposure
of the film emulsion revealed: (1) disruption of the epiphyseal plate, (2)
aseptic necrosis, and (3) osteosclerosis (Figure 13-B). These sequelae have
been described as early bone changes preceding long-term carcinogenic effects
from Sr-90 (30). These pathological syndromes (tumors and bone alterations)
are not pathognomonic of Sr-90 damage, but a correlation with the source
of radiation is conclusive.

SUMMARY AND CONCLUSION

Examples of radiation damage in this report have illustrated the wide
range of biological responses in mammals to ionizing radiations. Different
sources and types of radiations, dosage factors, species, and individual
differences make it necessary to detect the source of exposure before a
diagnosis of radiation damage can be stated with assurance. Familiarity
with the characteristics of radiation syndromes merely offers a means of
tentative diagnosis of radiation damage.

It is generally agreed that ionizing radiations are always harmful, yet
radiation damage is largely assessed by morphological criteria. Diagnostic
tests similar to those commonly employed in medicine have not been developed
to detect a biological response to ionizing radiation. Sensitive instruments
are available to detect the source of radiation, but, by comparison the methods
available for detecting radiation damage are only crude.

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23. See 2.


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QUESTIONS AND ANSWERS—CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

Charleston, West Virginia
October 18, 1960


DOCTOR ANDERSEN: The role of bacteremia in radiation sickness is well established even though the actual finding of bacteria in animals succumbing to irradiation may be difficult. In general, a wide range of bacteria have been found in lethal cases and the presumed portal of entry is via the gut. If irradiated animals are given antibiotics, survival is enhanced but not assured. In other words, antibiotics are not a specific therapeutic measure for radiation sickness, but their use increases chances of surviving.

DOCTOR H. W. DUNNE: Since virus infections also produce leukopenia, I wonder whether you could rule out viruses as a secondary factor.

DOCTOR ANDERSEN: An animal harboring a viral or bacterial agent and exposed to radiation may succumb, and the exact cause of death would be difficult to determine. For example, a sublethal radiation exposure could facilitate death in an animal harboring a virus disease. The radiation exposure merely lowered the animals' resistance as an adjunct in causing death from the viral infection. The relationship between pathogenic organisms and radiation exposure is a problem and helps to explain the LD 50/30 variation in the same species from different laboratories. Of the lesions shown this morning in radiation exposed animals, I am certain that all of us have at one time or another seen similar pathological effects caused by factors other than radiations. In other words, an absolute diagnosis without a history of radiation exposure creates a problem and one that I bring to your attention in this paper.

DOCTOR EGGERT, Ames, Iowa: Can an X-ray film be used in the intact animal to diagnose a source of radiation?

DOCTOR ANDERSEN: You probably have reference to an internal emitter, because an external source can readily be detected by X-ray film. Most laboratories use X-ray film badges to monitor exposure to personnel. However, with most internal emitters, the animal absorbs the radiation energy decreasing the effectiveness of X-ray film for detection of the source. Only the more energetic and gamma emitting radioisotopes can be detected by X-ray film. Sensitive electronic devices are used to detect internal emitters. For example, whole-body counting facilities—an iron room with special detectors—are commonly employed to detect and measure Sr$^{90}$ and other radioisotopes in humans and animals.
IDENTIFICATION OF *ESCHERICHIA COLI* SEROTYPES ISOLATED FROM DISEASES OF ANIMALS

PAUL J. GLANTZ, PH.D.

Diarrheal diseases of animals may be caused by a variety of microorganisms. The role of Shigella, Salmonella, Clostridia, and some viral agents in such cases is well-known. There are, however, many occasions when only *Escherichia coli* is isolated.

It is not unusual that these coliform bacteria caused so much controversy, since they were considered normal inhabitants of the intestinal tract. Many earlier workers were convinced of the pathogenicity of *E. coli*. A like number despaired at ever actually proving this concept since morphological, cultural, and biochemical characteristics were inadequate. Serological methods in use in earlier times gave inconsistent results. Kauffmann (3), by elucidating the characteristics of the K antigens, explained some of the causes of these inconsistencies and provided a basis for more precise studies on the serology of the *E. coli* group. His work was confirmed and extended by Ewing et al. (1), and others. This led to the establishment of a diagnostic coli-antigenic-schema, in which *E. coli* are classified according to their O (somatic), K (envelope or capsule), and H (flagellar) antigens.

The purpose of this paper is to discuss the methods used for the serological identification of *E. coli* strains isolated from diseases of animals. However, before serological classification can be carried out it is imperative to determine that the culture is *E. coli*, because there are many known intergeneric antigenic relationships.

Differential plating media that are not too highly selective, such as violet red bile agar (VRB), MacConkey agar, or eosin methylene blue agar (EMB) may be used. In addition blood agar or veal infusion agar plates should also be used since some *E. coli* strains grow more readily on these media.

Well isolated colonies should be transferred to culture media to determine their biochemical reactions. With a little experience one can note that while all coliform colonies on a violet red agar plate may seem to look alike, there are some differences. There may be solid red forms, red center with transparent edges, some may have entire edges, others may be indented. It is not to be implied that each different colony type may be a different serotype. However, since more than one colony is usually transferred, some method of selecting the colonies should be used.

The biochemical reactions which characterize typical *E. coli* strains were presented in a previous paper (2). It should suffice to mention that acid and gas from glucose, acid from lactose and sucrose, indole +, methyl red +, Voges Proskauer —, and a negative reaction for H₂S, urease, and Simon's

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citrate are typical for *E. coli*. It should be noted that many *E. coli* strains that are otherwise typical may fail to ferment lactose rapidly and anaerogenic cultures are not uncommon.

Once it is established that the culture belongs to the *E. coli* group, serological analysis may be made. Complete serological identification of the O, K, and H antigens of *E. coli* strains is a time consuming and rather involved procedure. However, preliminary identification can be done if suitable antisera are available. Since an understanding of the characteristics of the O, K, and H antigens is required before any serological procedures can be carried out, a brief resume appears in order.

The O antigens are thermostable and are not inactivated by heat at either 100° C. or 121° C. for 2 hours. The H antigens are thermolabile, being inactivated at 100° C. The term “K antigen” is a symbol for either envelope or capsule antigens. There are three varieties of K antigens, L, A, and B. Table 1 lists the different characteristics of a B type of K antigen.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>O 119:B14</th>
<th>O 119</th>
<th>O 26:B6</th>
<th>O 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 119:B14</td>
<td>(K) living</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(O) 1 hour at 100° C.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O 26:B6</td>
<td>(K) living</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>(O) 1 hour at 100° C.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

A living suspension of O 119:B14 antigen agglutinates with O 119:B14 antiserum. This is due to the reaction between K (B14) antigen and antibody. The presence of the K (B14) antigen in this culture is also responsible for its O-inagglutinability with the O 119 antisera.

The masking or inhibitory effect of the K (B14) antigen is inactivated by heat at 100° C. for one hour. The O antigen is then free to react with the O antibody of the O 119:B14 and O 119 antisera.

In a similar manner, an *E. coli* strain with a different O and K antigen, O 26:B6, will react as listed on Table 1. There is no cross reaction between the O or K antigens of these two *E. coli* strains.

The reactions listed in Table 1 are referred to as O grouping or OB grouping, since these are the only antigens identified. This is not serotyping. *E. coli* O 119 is not a serotype but an antigenic complex, the fractions of which have to be determined along with H and K for complete serotyping.

If an *E. coli* strain contained an A variety of K antigen, then it would be necessary to heat the suspension at 121° C. for 2½ hours before O agglutination would take place. This is due to the relatively thermostable capsular nature of the A antigens. The L type of K antigen has properties similar to those of the B variety.
It should be noted that an *E. coli* strain may be found with or without K antigens, or with a K antigen different from that one usually associated with a given O group.

From this discussion of the *E. coli* K antigens it should be apparent why the early investigators had so much difficulty in their attempts to classify *E. coli* cultures by serological methods using antisera prepared with whole, untreated cultures.

The methods for preparing the O, K, and H antigens of *E. coli* and the procedure for conducting the agglutination tests are those reported by Ewing *et al.* (1). It is very important that the *E. coli* strains utilized for serum production consist of smooth cultures since rough types cannot be used. Typical smooth colonies are readily apparent when grown on thick, fresh infusion agar plates. Rough types look rough and also form an agglutinate or coarse, flocculent precipitate when heated at 100° C. for 20 minutes or longer.

The O antigens are prepared from fresh, heavy broth cultures which are heated at 100° C. for one hour. After cooling they are diluted to a suitable density with five-tenths percent formalinized saline. The antigens are added to the O antiserum dilutions in tubes, mixed, and placed in a 50° C. water bath overnight. The reactions are then recorded.

The K antigens are also prepared from fresh, heavy broth cultures, but are not heated. They are diluted with formol-saline and then added to dilutions of K antisera, mixed, and placed in a 37° C. incubator for two hours. They are then placed in the refrigerator overnight. In contrast to the granular O agglutinate, positive K agglutination consists of a pellicle or a tight button of bacteria on the bottom of the tube.

To produce H antigens it is necessary to have maximum development of the flagellar forms. This may be accomplished by passing a culture through semisolid motility medium until rapid growth to the bottom of the tube is obtained. Broth cultures can then be prepared as mentioned above. The H antigens, however, are used in a heavier suspension than the O or K antigens. After being added to H antisera and mixed, the tubes are placed in a 48-50° C. water bath. The reactions, which consist of a fluffy, coarse agglutinate when positive, are read at 15-minute intervals for the first hour. The final reading is made one hour later or after a total of two hours incubation. In typical O agglutination the particles or aggregates remain in suspension when shaken. Those produced in H reactions will readily disperse, but will promptly agglutinate again within 15 minutes.

It should be realized that an O antigen, after heating at 100° C. for one hour, may have its A antigen intact, if this is present. An H antigen also could have its O and K antigen present. The K antigen in turn could have the H and O antigens of the parent strain.

The methods for carrying out the O, H, and K agglutination tests have been designed to eliminate, insofar as possible, reactions which might be due to the presence of an antigen other than the one being tested. The H, and O inagglutinable forms for K titrations, are stopped after two hours for it has been found that after this time O agglutination starts to become evident. The
IDENTIFICATION OF ESCHERICHIA COLI SEROTYPES

ultimate would be to have selectively absorbed pure O, K, and H antisera. Work along this line has already been done at the Communicable Disease Center in Atlanta, Georgia, and is being started at Penn State.

The procedure for serological identification of *E. coli* strains may vary according to the number and types of antisera to be utilized. If all of the 146 O antisera are available then it simplifies matters to make up seven or eight antisera pools. Each pool contains those O antisera which usually have some common antigenic characteristics. An unknown antigen is tested against a 1:500 dilution of the eight antisera pools. The antigen is then tested against a 1:500 dilution of each of the specific O antiserum components of those pools which gave a positive reaction. Each specific O antiserum giving a positive result is then tested in serial dilutions from 1:80 to 1:10,240 to determine the highest titer at which the *E. coli* antigen is agglutinated. Finally, cross absorbed serum may be used in a slide agglutination test to identify the specific O group to which the antigen belongs, and to determine O antigen fractions or sub-O-groups.

The H and K antigens may be determined in a similar manner if these sera are available.

For those who wish to search only for a few specific serotypes, slide agglutination is the most rapid method for preliminary work. The *E. coli* antisera, usually O and OK, can be kept in small dropper bottles for ease in dispensing drops on a slide. Suspensions of the *E. coli* cultures can be prepared and tested before and after heat treatment. It is also possible to pick colonies with a needle directly from a plain or blood agar plate culture and mix with the antisera on the slide. If, however, a colony contains a very large amount of K antigen, the suspension technique is recommended, since it is possible for the K agglutinate to clump on the inoculating needle tip while mixing with the antisera.

Reactions obtained on the slide are always confirmed by using serial dilution of the antiserum in tubes. This is necessary since the antiserum used for slide agglutination is tested at a dilution of 1:5 or 1:10. Cross reactions can and do occur at this low dilution, hence one must test further.

The question naturally arises whether serological typing of *E. coli* is practical for the average laboratory. Complete serotyping definitely is not. However, the O and K antigens of the *E. coli* strains most frequently associated with diseases of animals could be readily identified in a preliminary way at least. The antisera required for such typing should be obtained from one reliable source which emphasizes quality rather than quantity of its product.

At present, more emphasis should be placed on the research phases of *E. coli* serotyping. It is evident that once an *E. coli* strain has been identified serologically, we have a bacterium with a license plate which can readily be traced through various experiments *in vivo* or *in vitro*. With the specific antiserum for this bacterium we can search further in diseased and in healthy animals for evidence as to whether or not it is a pathogen. Such evidence will be found in outbreaks or epidemics, not in a few isolated cases. It is
important to remember that the more *E. coli* strains that are identified serologically, the better understanding we will have of their role in animal diseases.

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A group of viruses that inhabits the intestinal tract of man and animals has been classified as enteroviruses (Sixth International Congress of Microbiology, Rome, 1953). Most of these viruses are small spherical particles that pack into crystalline lattices; they contain mostly protein and ribose nucleic acid (RWA), are infective for suckling mice, and are resistant to environmental changes. The polio, Coxsackie, enteric cytopathogenic human orphan (ECHO), enteric cytopathogenic bovine orphan (ECBO), foot-and-mouth disease viruses, and perhaps others, clearly fall into this classification. Numerous agents about which less is known probably also are members of this group.

This paper will summarize some of the recent data on viral agents isolated from the alimentary tract of cattle which seem to fulfill at least some of the present requirements for classification as enteroviruses. Because so little is known about many of these agents, later studies may reveal that they do not belong in this group. Furthermore, it is likely that the rather empirical criteria for classification of viruses will continue to change.

The known data on some of the bovine enteric viruses that clearly do not fall into the present classification of enteroviruses will also be summarized. This paper, in consequence, would more properly be titled enteric viruses of cattle.

Special emphasis will be placed upon the aspects of these viruses that are of greatest importance to the virologist engaged in diagnostic work. Although foot-and-mouth disease virus has been classified as an enterovirus, it will not be included in this discussion because of its exotic nature.

**ENTERIC VIRUSES NOT DEFINITELY ASSOCIATED WITH A CLINICAL DISEASE**

*Enteric cytopathogenic bovine orphan (ECBO) viruses.*—After it was discovered that bovine serum may contain a substance that neutralizes poliomyelitis virus, several investigators embarked upon a systematic search, utilizing cell culture systems, for avirulent viral agents that might be useful as live poliomyelitis vaccines. Klein and Earley (4) isolated a total of 34 viruses in bovine embryonic kidney cell cultures from cattle feces and a latent virus from a calf kidney used to prepare a cell culture. They segregated these viruses into three groups on the basis of the type of cytopathic effect they produced. These agents were serologically unrelated to polio virus and their relationship to other viruses of animal and human origin was not reported.
On continuation of their studies, Klein et al. (5), found that the cytopathic virus isolated in bovine kidney cell culture from feces of a normal cow was neutralized by pools of human gamma globulin, and also by 35 percent of individual human serums tested. This agent could be propagated at a low titer in bovine kidney and testicle cell cultures, and less satisfactorily in monkey kidney cell cultures, but not in HeLa cells, chick embryos, or adult or suckling mice. It did not hemagglutinate human O, bovine, sheep or guinea pig red blood cells. Neutralization of the virus could not be demonstrated with batteries of antiserums against adeno, Coxsackie, ECHO, influenza, herpes simplex, virus B, mumps, polio and hemadsorptive viruses. The authors' conclusion was that this agent is antigenically related to some virus which commonly infects humans.

Kunin and Minuse (8) isolated eight filterable agents or perhaps, because they showed them to be antigenically related, eight strains of a filterable agent, from apparently healthy dairy cattle. These agents were cytopathic for bovine and monkey kidney and chicken embryo cell cultures. They were propagated in embryonated chicken eggs and produced paralysis and death in day-old mice. The pathogenicity of these agents for cattle was not demonstrated; and they were not associated with any disease process. These agents were not serologically related to polio, Coxsackie A or B, ECHO, vesicular stomatitis, calf pneumonia enteritis, bovine mucosal disease, or infectious bovine rhinotracheitis viruses. According to Kunin, Sabin also has isolated a cytopathogenic virus from the feces of a healthy calf.

McFerran (11) reported on 112 cytopathic virus isolates from feces of apparently normal cattle in Ireland. These agents were segregated into three sero-types. On further investigation one of the sero-types was found to be antigenically indistinguishable from one of Kunin's ECBO viruses. No attempts to investigate the pathogenicity of these viruses in bovine species were reported.

Moll and Davis (13) recovered six cytopathic viruses from feces and fetal fluids of animals with histories of respiratory disease and abortion. All produced striking cytopathic changes in bovine kidney cell cultures. At least one of the agents behaved similarly in swine, human, and monkey kidney cell cultures as well as in HeLa cell and human amnion cultures. These viruses were grouped into two sero-types which were found to be antigenically unrelated to infectious ulcerative stomatitis, bovine diarrhea, bovine agent X, polio, and adeno viruses, but were neutralized with infectious bovine rhinotracheitis and Kunin's ECBO antiserums. In animal experimentation with one of the agents, infectivity was not demonstrated in suckling or weaned mice, nor in normal calves. Myocarditis was observed in cortisone treated mice, and lung consolidation in cortisone treated calves, but virus could not be isolated from the affected tissues.

Moll's calf virus.—Moll and Finlayson (12) isolated a cytopathic virus from the feces of cattle that exhibited clinical signs similar to the viral diarrhea-mucosal disease complex. No serological comparison between this agent and viral diarrhea virus was reported.
Light's hemorrhagic enteritis virus.—Of particular interest from the epidemiological point of view is the report of Light and Hodes (9) concerning the isolation of a viral agent from diarrheal stools of infants. This virus produced a hemorrhagic diarrhea when inoculated into calves. It was not identified as being related to any known bovine virus.

As far as we have been able to ascertain, with the exception of infectious bovine rhinotracheitis, the relationship of the ECBO viruses to viral agents that are pathogenic for the bovine respiratory system has not been determined. One would expect that some of them also inhabit the bovine respiratory system.

At the present time, only some of the ECBO viruses can be identified by means of serum neutralization in tissue culture. Undoubtedly as research progresses, diagnostic tests will become available for the identification of these agents. It is also possible that an additional diagnostic aid may be found in the cold hemagglutination technique because, as reported by Mascovici and Maisel (10), five out of eleven ECBO strains tested agglutinated bovine red blood cells, and three strains agglutinated guinea pig cells when the tests were carried out at 5 C.

BOVINE ENTERIC VIRUSES OF KNOWN PATHOGENICITY

Calf pneumonia enteritis.—In 1943 Baker (1) isolated a viral agent from field cases of calf enteritis which produced pneumonia and enteritis in experimentally inoculated calves. He adapted the agent to mice and demonstrated that neutralizing antibodies occur in the serum of convalescent animals. Calf pneumonia enteritis is characterized by yellow watery diarrhea, and a very mild interstitial pneumonia which ordinarily occurs within one to three days of age. The virus is transmitted experimentally by aerosol (2). Adult cattle have been found to harbor the virus in their lungs. Calves over 48 hours of age are highly resistant to infection.

Calf pneumonia enteritis virus is a spherical body approximately 20 µ in diameter (2). It has been propagated in gravid uteri of mice and guinea pigs, as well as in the calf. The virus has not been propagated in cell culture. No diagnostic tests other than animal inoculation procedures have been developed for calf pneumonia enteritis.

Viral diarrhea-mucosal disease complex (VD-MD).—This syndrome is an acute or chronic contagious and infectious disease of cattle characterized by fever, nasal discharge, cough, diarrhea, dehydration, leucopenia, erosions and inflammation of the mucous membranes of the alimentary canal, and swelling of the lymph nodes. Although severe forms of the disease never have been produced experimentally and much remains to be learned about the pathogenesis of the viral diarrhea-mucosal disease complex, it is quite generally conceded that this syndrome is caused at least in part by a virus.

VD-MD virus has been propagated in rabbits, sheep, young swine and cell cultures. Preliminary results indicated it is probably a spherical particle of about 65 µ in diameter. The virus can be isolated from the feces of chronic
cases for many months. Numerous cytopathic and non-cytopathic isolates have been identified.

Cross protection tests in cattle and serum neutralization tests in cell cultures indicate most of the isolates of viral diarrhea-mucosal disease viruses antigenically are similar (3) (6). The only published evidence of the existence of immunologically different strains was the report of a strain isolated in Indiana (VD-143) that failed to protect against viral diarrhea virus-strain NY on reciprocal cross protection tests (15). This strain of viral diarrhea virus unfortunately no longer is available. This may be the reason that convalescent serums from some animals that exhibit characteristic clinical and pathological signs of the viral diarrhea-mucosal disease complex do not contain high titers of neutralizing antibodies for the cytopathic viral diarrhea virus.

Animal inoculation and serum neutralization tests in cell cultures, using a cytopathic isolate of VD-MD virus, are the only practical diagnostic procedures available.

*Miyagawanella bovis.*—York and Baker (18) isolated a virus belonging to the Psittacosis-Lymphogranuloma group from the intestinal tract of apparently normal calves from six herds examined. The virus was propagated in guinea pigs and embryonated chicken eggs. It produced pneumonia in mice and a response in cats, rabbits and swine. They named the virus *Miyagawanella bovis.*

Extensive studies have been conducted in Japan during the last decade on natural and experimentally produced *M. bovis* infections in cattle. Much of this work recently was summarized by Omori *et al.* (14). The Japanese work suggests that *M. bovis* infection in cattle is a systemic infection, with the most marked lesions occurring in the nervous and respiratory systems. They demonstrated that a high proportion of recovered animals were fecal carriers. These workers believed that the agent they were working with closely resembled York's agent and the causative agent of sporadic bovine encephalomyelitis (17), as well as goat pneumonitis virus, sheep pneumonitis virus, sheep abortion virus, and cat pneumonitis virus.

*M. bovis* elementary bodies readily can be identified in smears from infected yolk sacs stained with basic dyes (Giemsa's and Macchiavello's) with an ordinary light microscope. They are more difficult to demonstrate in tissues and exudates of animals. The virus is a spherical particle 300-400 μ in diameter, with a dense irregular center. It is relatively resistant to environmental changes and remains infective for at least a month at 4 C. and at least a year at -70 C. It is more or less susceptible to a large number of antibiotics. Intracytoplasmic inclusion bodies can be demonstrated in tissues.

Virus isolation, preferably in the guinea pig and embryonated chicken egg, complement-fixation tests, and pathological examinations all are helpful in making a diagnosis of *Miyagawanella bovis* infection.
CONCLUSIONS

It would appear, therefore, from the foregoing that the bovine alimentary canal is a fertile locus for the propagation of various viruses cytopathic for cell cultures. Undoubtedly many more viruses will be isolated as the search continues. It would seem logical to assume that numerous other viral agents, neither pathogenic to the host nor cytopathic to cell cultures, also probably inhabit the bovine alimentary tract. The isolation and identification of these agents must await the development of a method to detect non-cytopathic and non-pathogenic viruses. Perhaps a procedure based on the virus interference phenomenon as was used by Kniazeff and McClain (7) for the quantitation of the non-cytopathic viral diarrhea virus will fulfill this requirement.

Considering the already obvious similarity between the bovine and human enteric viruses, and the extremely broad pathogenic implications of the human enteric virus infections (16), it is certain that the bovine enteroviruses will present challenging problems to animal disease investigators.

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INCIDENCE AND ECONOMIC EFFECTS OF ENTEROVIRUS IN IOWA SWINE

WILLIAM P. SWITZER, D.V.M., PH.D.

Ames, Iowa

Enteroviruses have been observed to occur in swine by several workers (1, 2, 3, 4, 5, 6, 7, 9, 12 and 13). Information is still unavailable as to the incidence and economic effects of these viruses. This work was undertaken to determine the incidence of enteroviruses in young Iowa swine and the effect such viruses exerted on the feed efficiency of these pigs.

METHOD OF PROCEDURE

Four pigs from 40 to 60 pounds in weight were available for repeated sampling from each of 52 Iowa herds. In a few instances more than one group of pigs was available from a given herd. These pigs originated from among the more progressive swine breeding establishments in the state. These pigs were housed in uniform pens and fed identical rations while under the supervision of animal nutrition personnel at a terminal point.

Sterile cotton swabs were used to collect feces from the rectum of two of the four pigs in each group. These samples were collected at the time the pigs were unloaded from the owner's truck at the terminal point. The swabs were returned to the laboratory a few hours after collection. The two samples from each group were pooled, suspended in two ml. of balanced saline A (8) containing 2,000 units penicillin, 1,000 mcg streptomycin, 250 mcg erythromycin and 100 mcg mycostatin per ml. After one hour treatment at room temperature the suspension was either processed immediately or stored at —20°C for one to five days. Prior to inoculation of the samples into cell cultures they were centrifuged at 2,000 RPM for 10 minutes. One-tenth ml. of supernatant fluid was inoculated into each of four tubes of primary swine kidney cell cultures. These cell cultures were grown from kidneys collected from pigs one to six weeks old. The methods used to grow and maintain the cell sheets have been described previously (11). Five to seven days after inoculation of the fecal sample into the cell cultures the fluids were harvested and passaged in a second series. All viral isolates were serially passaged through at least four passages in cell cultures and were checked for bacterial sterility by inoculation on horse blood-tryptose agar.

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The cooperation and assistance of Dr. M. Spear and his associates in sample collection and evaluation of the performance of the animals is gratefully acknowledged.
After each group of pigs had been in individual pens at the terminal point for approximately 45 days a second collection of rectal swabs was made. Two pigs were sampled from each group of four pigs but no attempt was made to select the same two pigs collected from initially. The feed efficiency of these groups of pigs were secured for the approximate interval between samplings.

RESULTS

Rectal swabs were collected from 52 different groups of pigs. Primary swine kidney cell culture cytopathogenic agents were recovered from 15 of these groups upon arrival at the terminal.

In a previous report (12) an attempt was made to divide swine nasal virus isolates into types 1, 2 and 3 on the basis of the type of cell destruction produced. Seven of the 15 enteric virus isolates recovered in the present study produced changes typical of type 2 cell destruction while eight isolates produced cell destruction typical of type 3 cell changes. The second set of rectal swab samples, collected approximately 45 days after the first, yielded six cytopathogenic virus isolates. All six of these isolates produced type 3 cell destruction. It was of interest that only one group of pigs yielding an enteric virus isolate upon initial sampling had virus present at the time of the second sampling. In this one case the initial virus isolated produced type 2 cell destruction while that isolated approximately 45 days later was a type 3 cell destruction virus.

In none of these groups of pigs was there a definite diarrhea except in one pen of pigs yielding a type 2 cell destruction virus upon initial sampling. This group of pigs was unusually excitable and evidenced a diarrhea for about two weeks after their arrival.

A comparison of the feed efficiency of the groups of pigs for the approximate period between samplings failed to show any significant difference at the 95 percent confidence level between enteric virus infected and noninfected groups by either the F test or the T test (10).

DISCUSSION

The finding that approximately 30 percent of the groups of young swine sampled, harbored detectable enteric viruses was unexpectedly high. The sampling technique probably failed to demonstrate all infected groups and some groups may have had prior infection and eliminated the virus. These groups of swine originated from among the more progressive swine breeding establishments in the state. Therefore the occurrence of virus may be biased toward a lower incidence.

It was not possible to correlate any clinical disease with the occurrence of these enteric viruses in the pigs. No history was available to indicate if mild enteric infections were a problem in younger pigs on the farms of origin.

The presence of enteric viruses had no detectable effect on the feed efficiency of the infected groups of pigs. It is believed that feed efficiency
gives a more critical evaluation of the effects of mild enteric infections than does rate of gain since the possibility of the pigs eating an increased amount of feed to compensate for mild diarrhea is eliminated.

SUMMARY

Enteroviruses were isolated from approximately 30 percent of 52 groups of 40 to 60 pound Iowa pigs. These viruses produced two different types of cell destruction in primary swine kidney cell cultures. When these same groups of pigs were sampled approximately 45 days later all but one of the infected groups were negative. However six additional groups were infected. The presence of enteric viruses had no significant effect on the feed efficiency of the infected groups of pigs. No detectable clinical disease was associated with these enteric viruses.

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DIFFERENTIAL DIAGNOSIS OF THE MORE COMMON INFECTIOUS OVINE ABORTIONS OCCURRING IN THE UNITED STATES

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The more important differential diagnostic features of the foremost commonly occurring infectious ovine abortion conditions in the United States will be discussed in the material to follow. The problems referred to are vibriosis, ovine virus abortion, listeriosis and toxoplasmosis.

VIBRIOSIS

Autopsy Procedure

Material from the stomach and liver is cultured in duplicate tubes of Albulmi Brucella broth to which 0.15 percent of agar has been added to make a semi-solid medium. Usually, seven percent of sterile horse serum is added to each tube after sterilization. Fluid from the stomach or bits of liver tissue may conveniently be obtained by searing the surface of the organ with a hot iron and picking up samples with a rubber bulb-equipped Pasteur pipette. The heart blood of the fetus is sometimes cultured but vibrios are seldom obtained from this source. If a portion of the fetus has been destroyed by scavengers the brain should be cultured as it frequently yields Vibrio fetus and is protected from damage and consequent gross contamination by the skull.

At the time of autopsy gram-stained smears of the stomach fluid are examined for vibrios. Wet preparations may be examined by phase contrast. Many fetuses which are negative for vibrios on microscopic examination prove to be positive on culture. If the smears are negative, tissue should be frozen for possible future egg inoculation for the isolation of the virus of enzootic abortion. If cotyledons are available, impression smears should be stained with the Stamp modification of the Ziehl-Neelsen stain and examined for elementary bodies; smears may also be stained with the Krajian silver stain and examined for the presence of leptospira.

Cotyledons as well as brain, liver, and heart tissue should be placed in 10 percent formalin in the event that an examination for toxoplasma is desired. Sections may also be stained and examined for the presence of leptospira.

Examination of Cultures

Stained smears may be made of the cultures after 48 hours incubation under an atmosphere of 15 percent carbon dioxide, but the cultures should not be discarded before they have been incubated four days. Growth of

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**DIFFERENTIAL DIAGNOSIS OF OVINE ABORTIONS**

*V. fetus* occurs in a narrow zone at the top of the semi-solid medium. Cultures which are found to be positive for vibrios are streaked out on blood agar plates and picked cultures are checked for catalase, and shows growth at or just beneath the surface of deep stab cultures. Occasionally, vibrios other than *V. fetus* may cause outbreaks of abortion.

The medium employed and the conditions of incubation are suitable for the growth of nearly all bacterial agents associated with abortion, with the exception of leptospira. In addition to *V. fetus*, ovine abortion may be caused by brucella, salmonella, listeria, toxoplasma, leptospira, and the virus of enzootic abortion.

**OVINE VIRUS ABORTION**

Ovine virus abortion is characterized by abortion or birth of weak lambs that soon die or apparently normal lambs that die soon after birth.

The fetal membranes and cotyledons appear to be the only tissue susceptible to the virus. Gross examination reveals varying degrees of necrosis of the cotyledons and chorion with an accumulation of a dirty reddish discharge. The affected cotyledons lose their normal dark purple color and appear dark red to a pale color. These infected cotyledons are firmer in consistency than normal and have a matted appearance. The extent of cotyledon and membrane infection varies from case to case. The chorion shows thickened areas ranging from an edematous to a dry leathery tissue. The extent of these areas varies from case to case.

Post-mortem examination of aborted fetuses from field cases show varying degrees of blood-tinged edema in the subcutaneous and intramuscular tissues and the body cavities usually contain varying amounts of a blood-tinged serous fluid. The organs of many aborted fetuses and premature lambs are friable and pale in color.

**DIAGNOSIS**

A diagnosis of ovine virus abortion can be made by the complement-fixation test, fetal cotyledon impression smears, egg and animal inoculation.

For routine diagnostic purposes, smears of fetal stomach contents or fetal tissues are of little value since elementary bodies normally cannot be demonstrated microscopically. Placental tissues are frequently used to make a diagnosis of ovine virus abortion. Impression smears of fetal cotyledons and membranes may be made, stained by the modified Ziehl-Neelsen method.

In diagnosing ovine virus abortion by placental tissue smears, elementary bodies must be differentiated from contaminating bacteria since some bacteria will stain red with the modified Ziehl-Neelsen method.

Smears are examined microscopically for the minute pink to red staining elementary bodies, less than .5 micron in diameter, in epithelial cells, mononuclear and polymorphonuclear leukocytes. Elementary bodies are seen extracellularly in smears of fetal cotyledons and membranes.
The developing chick embryo is another diagnostic aid in this disease since it can be infected easily. The virus of ovine abortion can be isolated from infected placental tissues by cultivation in the yolk sac of chick embryos. The virus is seldom isolated in yolk sac cultures from any of the fetal organs. Cotyledons and membranes are ground, suspended in a diluent, TSS (antibiotic solution) added in cases of bacterial contamination, and the yolk sacs of five- to seven-day-old chick embryos inoculated.

Yolk sacs from infected embryos that die after 48 hours following inoculation are harvested, smeared, stained by the modified Ziehl-Neelsen method and examined for elementary bodies.

Since infected chick embryos may occasionally survive, yolk sacs from survivors should also be harvested and examined for elementary bodies.

In cases where fetal cotyledons and membranes are not available, elementary bodies may be demonstrated on microscopic examination of vaginal swab-smears taken soon after abortion. Negative vaginal swab-smears from a few aborting ewes in a flock do not constitute evidence that these abortions are not caused by the virus of ovine abortion.

Guinea pigs and mice may be used to diagnose ovine virus abortion. Guinea pigs are inoculated intraperitoneally with suspensions of infective ovine placental tissues, killed five to seven days after inoculation, and the organs collected. The virus of ovine abortion can be isolated by yolk sac cultures from suspensions of infected guinea pig lung, liver and spleen. The livers of infected guinea pigs may show minute lesions which appear grossly to be necrotic foci ranging from pinpoint to about two mm. in size.

The virus may also be isolated by yolk sac cultures of guinea pig fetal cotyledons. The sows, about 30 days in gestation, are inoculated with suspensions of infective ovine placental tissues and killed some 30 days later. The cotyledons are collected and suspensions prepared for yolk sac inoculation. Stained smears of guinea pig fetal cotyledons may show elementary bodies.

Mice can be infected by intranasal inoculation. This virus possesses the pneumotropic characteristics of some of the other members of the psittacosis lymphogranuloma group. Approximately half the inoculated mice may die five to seven days after inoculation and the lungs show varying degrees of consolidation. Elementary bodies usually can be demonstrated in stained smears made from these areas of consolidation.

Complement-fixation titers of 1:32 are generally considered positive and 1:16 suspicious. However, our experience has shown a 1:8 fixation to be indicative of infection.

A low complement-fixation titer may be present at the time of abortion or premature birth. Maximum titers occur between two weeks and four months after abortion.

DIFFERENTIAL DIAGNOSIS

Bacterial cultures should be prepared for bacterial agents of abortion especially *Vibrio fetus* and *Listeria monocytogenes*. Virus abortion can be
differentiated from the infectious bacterial abortions and nonspecific abortions by the complement-fixation test, smears of fetal cotyledons and membranes, inoculation of chick embryos, and mice or guinea pig inoculation with infective placental tissues.

**ISOLATION OF LISTERIA MONOCYTOGENES**

1. Since *L. monocytogenes* is often located intracellularly, tissue to be cultured should be macerated in a mortar, Waring blender, glass tissue grinder or similar device together with a few ml. of sterile distilled water or nutrient broth to make fairly thin suspension. (Saline should be avoided as it may have harmful effect especially if bacterial population is low.)

2. Plate several loopfulls of this suspension on tryptose agar (Difco) or modified McBride’s agar. (Blood plates may be used but lack advantage of step six. May show small zone of hemolysis.)

3. Body fluids, swabs, etc., plated as in step two.

4. After plating (Step two), tissue suspensions (Step one) and fluids, etc. (Step three) are kept at 4°C.

5. Plates are incubated at 37°C. for 18-24 hours.

6. After incubation plates are examined with scanning microscope (15), or hand lens with petri dish on laboratory tripod, using obliquely transmitted light. Colonies of *L. monocytogenes* are distinctive blue-green in color and with a little practice can be identified quickly even in badly contaminated cultures. Colonies are 0.2-0.8 mm. in diameter, round with an entire margin, translucent, slightly raised with finely textured surface, and watery in consistency.

7. If *L. monocytogenes* is not detected in Step six, refrigerated material (Step four) should be replated at intervals of several days for a period of two-to-three months. If no growth occurs during this time culture may be considered negative. Listeria infection *cannot be ruled out* merely on the basis of negative results on initial culture attempts. This fact is now well established, but has no explanation.

8. With the difficulty of isolation on nonliving media, material to be cultured could be inoculated I.P. into mice or on CA membrane of 10-day-old embryonating chicken eggs.
   A. Listeria infection in mice characterized by focal hepatic necrosis.
   B. Listeric infection in eggs characterized by conspicuous necrotic foci on CA membrane.
   C. *L. monocytogenes* usually isolated easily from mice or eggs.

9. Fluorescent antibody techniques desirable to avoid possible long period in confirming clinical diagnosis.
10. Identification of suspect cultures.
   A. Fermentation and biochemical tests.
   B. Motile at “room temperature” (18-20°C.) but usually not at 37°C.
   C. Production of monocytosis in rabbits or mice.
   D. Conjunctivitis in rabbits, guinea pigs or mice.

TOXOPLASMOsis

Toxoplasma caused prematurities in sheep have been a rarity in the United States. However, it should be considered. Encephalitic symptoms may be noted as well as abortions.

Serological, microscopic and animal inoculation tests may be used for confirmation of the infection. The relatively simple animal-inoculation procedures, in which white mice are used, depend on the appearance of toxoplasma organisms in the smear of peritoneal exudate of the test animals.
THE DIFFERENTIAL DIAGNOSIS OF BOVINE ABORTION

J. A. HOWARTH, D.V.M., PH.D.*

The dairy and beef industries are dependent on reproductive efficiency for the maintenance of their economic well-being. A dairy cow must conceive readily and deliver full-term calves during her useful life span in order to fulfill potential milking ability. Live healthy calves are also necessary for genetically desirable herd replacements. Living full-term calves are the basic product of the beef cattle industry. The economic necessity of selling non-calving cows prevents the selection of breeding cows on the basis of quality and age. Thus, herds experiencing abortion or low calving percentage will deteriorate in quality unless replacements are purchased.

Precise figures regarding losses due specifically to abortion are difficult to obtain. In dairy animals abortion may not be emphasized, particularly if a dead fetus is expelled near the end of the gestation period and milk production is only moderately affected. The 1956 report of the New Zealand Dairy Board (1) cites a 2.8 percent incidence of abortion in that country, but the same report states that only half of the actual abortions are reported. A four-year study by the British Research Council (2) of prenatal and postnatal calf losses states that 6.5 percent of all calves born were dead and that 56.4 percent of losses of calves up to six months of age were prenatal. A 1955 survey by the Research Committee of the American National Cattlemen's Association showed that only 80 percent of 200,000 cows studied produced live calves and 62 percent weaned calves. Here again, half the losses up to weaning time were prenatal.

To determine the cause of a low calving percentage in a given herd, the knowledge of both the clinician and the laboratory diagnostician is necessary. The poor conception rates and early abortions due to vibriosis and trichomoniasis must first be recognized by the clinician and then confirmed in the laboratory. In other instances full-term fetuses are traceable to management factors, as when heifers are bred too young and dystocia is common. Clinical and epizootiologic data are necessary for the evaluation of any abortion problem. The clinical history of the individual cow should indicate whether pre-abortion sickness, retention of fetal membranes, or post-abortion metritis is a common finding.

Data to be gathered for the herd history should include the number of abortions and the stage of gestation at which each occurred. The time interval encompassed by the outbreak is of utmost importance but it can be influenced by breeding schedules. In beef cattle an effort is made to have all cows calve within the shortest possible time, while in dairy herds calving may be spread over the entire calendar year. This one factor often influences the apparent

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explosiveness of the abortion storm. If pregnancy testing is a routine procedure for a herd, additional information can be gathered concerning unrecognized abortions. Such factors as seasonal incidence, age groups involved and repeat abortions in the same cow should be investigated. The type of terrain and available feed may give a clue to the possible etiology. The knowledge of the existence of abortion in several herds within an area at the same time is invaluable to the laboratory diagnostician.

The classification of bovine abortion on the basis of epizootiologic data is not only important to the clinician but should aid the laboratory diagnostician in his choice of laboratory procedures for identification of the etiologic agent.

CLASSIFICATION OF BOVINE ABORTION

I. Abortion of Epizootic Occurrence.
   1. Brucellosis
   2. Leptospirosis
   3. Epizootic Bovine Viral Abortion
   4. Lowland Abortion Syndrome

II. Abortion of Sporadic Occurrence with High Incidence Within Herd.
   1. Listeriosis
   2. Toxoplasmosis
   3. Salmonellosis

III. Abortion of Sporadic Occurrence with Low Incidence Within Herd.
   1. Vibriosis
   2. Avian Tubercular Infection
   3. Mycotic Infections

IV. Miscellaneous Agents Having Possible Significance in Bovine Abortion.
   1. Trypanosomiasis
   2. Cytopathogenic Enterovirus Infections
   3. Mucosal Disease
   4. Rift Valley Fever and Wesselsbron Virus Infections
   5. Modified Live Virus Vaccines
   6. Development of Erythrocyte Antibodies
   7. Bovine Dwarfism
   8. Poisonous Plants
   9. Deficiency Diseases

I. ABORTIONS OF EPIZOOTIC OCCURRENCE

This group includes those diseases having widespread distribution in a geographical area during a definite time interval and with heavy losses within the affected herds.
**DIFFERENTIAL DIAGNOSIS OF BOVINE ABORTION**

**Brucellosis:** The introduction of brucella infection into an area containing a high percentage of non-vaccinated cattle would undoubtedly result in an epizootic characterized by abortion. The rate of spread of such an infection would be influenced by the traffic of animals between herds, use of common facilities and sanitary practices. The advent of immunizing procedures and area-wide eradication programs has virtually eliminated the possibility of an epizootic of brucellosis in cattle in most areas. In California during the years of 1958 and 1959, brucellosis was found to be the cause of abortion in relatively few herds. However, until that time when brucellosis can be completely eradicated, it must be realized that brucellosis is a potential hazard and must be considered each time an aborted bovine fetus is presented to the diagnostic laboratory.

**Leptospirosis:** *Leptospira pomona* has been the serotype most commonly incriminated in bovine abortion. Other leptospiral serotypes have been implicated in abortion in various animal species and, therefore, must be suspected when serum samples are negative to *L. pomona* (3, 4, 5, 6).

Although Fennestad and Borg-Peterson (7) reported hemoglobinuria preceding abortion, it is most common for cows to abort without showing demonstrable clinical signs.

The isolation of leptospiras from fetal tissues by cultural methods and by animal inoculation has been reported (8, 9, 10). The demonstration of leptospira in silver stained histological sections from fetal tissues has also been reported (7, 11, 12, 13). However, other investigators after careful studies have failed to demonstrate leptospira in fetal tissues (14, 15, 16). Therefore, complete reliance should not be placed on this method for the diagnosis of leptospiral abortion. Serologic testing of the dam is still the most reliable diagnostic method. A representative number of samples should be examined and the diagnosis rendered on a herd basis. While serologic testing of acute and convalescent serum samples is the classical means of determining recent infections, it is common for cows to have leptospiral titers at the time of abortion. In the interpretation of results it must also be realized that non-aborting cows may have leptospiral titers.

Serologic results and the rate at which abortion occurs must be viewed in the light of management practices. The course of leptospirosis in a large dry-lot type of dairy operation may extend over the period of a year if immunization is not resorted to. During this period 60 percent of the cows may become serologically positive and approximately 20 percent may abort. Clinical sickness may be recognized in a small percentage and an occasional death may occur. In beef cattle housed under conditions of close contact an abortion rate of 30 to 40 percent may occur within a three-month period while the reactor rate may reach 60 to 70 percent.

In beef cattle maintained under western rangeland conditions where contact is minimal, leptospirosis assumes an entirely different character. The results of a study conducted in two rangeland beef herds having a 10 percent annual abortion rate are presented in Table 1. As judged by serologic testing, most cows which conceived and delivered live calves evidently escaped lepto-
spiral infection. Among the groups that either failed to conceive or had aborted, the rate of leptospiral infection was significantly higher. The two factors responsible for the limited spread of infection and distinct serologic pattern are the lack of close contact between cows and slaughter each year of cows which fail to calve. The testing of non-calving cows from rangeland beef herds having low calving percentage may yield additional information concerning the nature of leptospirosis.

Epizootic bovine viral abortion: For many years a form of bovine abortion in which no etiologic agent could be incriminated has been widespread in California. Howarth, et al. (17, 18), have reviewed the field aspects of the condition, described the pathology, and reported attempts to reproduce the disease experimentally. Because of the widespread occurrence of the disease in certain geographical areas, the high incidence within the herd, and its seasonal specificity, the term epizootic bovine abortion was suggested.

Studies to date indicate that the disease is restricted to animals which spend a portion of their gestation period in foothill or mountainous areas. Cows may abort in the foothills or after they have been returned to the valley. The most serious outbreaks of epizootic abortion have occurred when a herd of valley or imported cattle is sent to foothill pastures for the first time. In highly susceptible cattle such as these, abortion occurs in all age groups and the total losses may reach 60 percent. In succeeding years losses are usually confined to first calf heifers sent to mountain pastures for the first time. Consistent yearly losses of 30 to 40 percent in first calf heifers may result thereafter. In herds where all age groups are maintained in the foothills on a year-round basis, losses are negligible because animals are exposed to infection and become immune before breeding age. Thus, the disease is mainly restricted to herds that use foothill areas for spring pasturage. Abortion losses are recognized from July through November, with the time of occurrence probably influenced by differences of pasture season in various parts of the state. A relationship between the calving date and abortion losses has also been established. Greatest losses occur in herds using the commonly practiced calving season of October to January, while herds which calve during the April to June period sustain only minor losses. Animals calving during this later period are exposed to infection, but this exposure is near calving time and does not result in abortion.

Since the first published report of this disease, additional observations on the pathologic changes have been reported by Kennedy, et al. (19). No signs of impending abortions have been noted in the herds observed. Well-developed fetuses aborted during the last trimester of pregnancy are presented to the laboratory for examination. They usually show no discoloration or autolysis as fetal death probably occurs just before or during delivery. Petechial hemorrhages are regularly seen in conjunctival and oral mucous membranes. Straw-colored pleural and peritoneal effusions are common; in some cases the ascites is sufficient to produce a distended abdomen. The subcutaneous tissues are edematous, particularly those of the head. In most cases the striking gross lesion in these fetuses is the swollen, coarsely nodular liver.
This lesion was stressed in the original report of the disease and is frequently the most useful diagnostic lesion. This finding has recently been described in aborted bovine fetuses in Germany (20). Petechial hemorrhages are usually present in the mucosae of the trachea and esophagus. Small gray foci are irregularly distributed in the ventricular myocardium and in the renal cortices. The basic histological change of this disease is a granulomatous inflammatory process involving the mesenchyme of all body organs and is most easily recognized in the spleen and lymph nodes.

A large elementary body-producing viral agent has been isolated from aborted fetuses by Storz, et al. (21). Several isolates were obtained from fetal tissues and fluids inoculated by the yolk-sac route into hen eggs. The agent appears to be a member of the Psittacosis-LGV group of viruses. Parenteral inoculation of the virus into cattle produced a marked febrile response, followed by abortion after a period of 33 to 126 days. Pathological findings in the fetuses that were aborted following experimental inoculations were identical to those found in fetuses from field outbreaks. In view of the consistency with which the virus produces abortion in experimental cattle, it appears almost certain that it would be capable of causing the same effect under field conditions.

It is known that a member of the Psittacosis-LGV group of viruses is the cause of enzootic abortion in ewes. Stamp, et al. (22), showed that the ewe abortion virus can cause cattle to abort. Boulanger and Bannister (23), who inoculated cattle via the teat canal with the agent of Stamp, confirmed this finding. Schoop and Kauker (24) suggested, on the basis of smear preparations and serological studies, that a form of abortion they studied in Germany is caused by a member of the Psittacosis-LGV group of agents.

With regard to the identity of the agent isolated from epizootic abortion, present studies indicate that it is not Miyagawanella bovis or the agent of sporadic bovine encephalomyelitis.

Lowland abortion syndrome: A new type of bovine abortion has been described by Simon, et al. (25), in Wisconsin called the "Lowland Abortion Syndrome." They have described up to 400 abortions in beef and dairy cattle on unimproved lowland pastures in one county alone. No infectious agent has been found. The incidence was highest in May, June and September. As it appeared to be associated with weeds, a controlled experiment was set up and half a pasture was sprayed with hormone weed-killer. On the unsprayed pasture 10 of 12 pregnant heifers aborted, but on the sprayed part only one of eight aborted. The flora of the pastures varied but a number of the weeds found had high nitrate and nitrite content which the authors postulated was responsible for the abortions, although methemoglobin was not found in blood samples which were collected at weekly intervals.

No signs of impending abortions were observed. Fetuses appeared to have been dead some time and were found to have 100 ml. to 1,000 ml. of serosanguineous fluid in the pleural and peritoneal cavities. The heart showed degeneration of muscle and petechial hemorrhages. The pleura of both lungs was thickened. There were degeneration and foci of necrosis in the liver.
and necrosis of the spleen. The medulla of the kidney was congested and necrosed. In eight of 10 cases the fetal membranes were edematous with characteristic intercotyledonary, circumscribed, necrotic calcified lesions.

Laboratory confirmation of the diagnosis of abortion due to nitrate intoxication entails a determination of methemoglobin level in the blood of the dam or fetus or a nitrate ion determination in protein-free filtrates of fetal blood or tissues, the dam's blood or in feedstuffs (26).

II. ABORTION OF SPORADIC OCCURRENCE WITH HIGH INCIDENCE WITHIN THE HERD

Listeriosis: The early reports in the literature on bovine abortion due to listeriosis are mostly confined to isolations of the organism from one or two fetuses (27, 28). The more recent reports of Young and Firehammer (29) and Osebold, et al. (30), however, point out that a high incidence of abortion within a herd may occur even though the number of herds involved at any one time may be of a low order. Bovine listerial abortion differs from most other bovine abortion diseases in that clinical sickness of co-ws may be evident during the outbreak. Young and Firehammer (29) described the clinical picture as one of depression but not encephalitis. Of the 250 cows involved, 21 aborted and six died. In the three herds studied by Osebold, et al. (30), the disease was characterized by abortion, fever in the dam, frequent retention of fetal membranes and metritis.

If fetal death occurs near the time of abortion and autolysis is minimal, the most consistent histological change is focal necrosis of the liver (31, 32). For isolation of the organism from the aborted fetus the tissues of preference are lung, liver, spleen and stomach contents. It is very important to reculture the original materials after refrigeration at 4° C. for six weeks. A greater percentage of isolations is accomplished by this method (33). The organism can be isolated from uterine exudates collected soon after abortion, particularly if the reculture technique is used. No adequate serologic method has been developed, although the antigen-fixation test shows some promise (33).

Toxoplasmosis: Although reports of the in utero transmission of toxoplasma in the bovine are sparse, reports of such invasion of the uterus in other species are common (34, 35, 36, 37). Toxoplasmosis is regarded as one of the primary causes of ovine perinatal mortality in New Zealand with losses in some flocks ranging from three to 50 percent (38, 39). Because of this well-established incidence in sheep and because of the report of the disease in cattle, toxoplasmosis must be considered as being capable of causing severe losses in cattle.

While the pathology noted in aborted lambs is not distinctive, that which occurs in the fetal membranes as reported by Hartley, et al. (38), is distinctive. Fetal cotyledons tended to be convex, bright to dark red in color, with numerous white flecks or small soft white nodules. It is expected that similar pathologic changes would occur in the bovine disease.

Laboratory confirmation of toxoplasmosis should include Giemsa staining of impression smears and tissues from the placenta and fetus. To isolate the
organism, antibiotic-treated emulsified tissues are inoculated into mice. After a suitable interval, the peritoneal exudates of mice are stained by the Giemsa method and the mice serums are checked for antibodies with dye test (40). Circulating antibodies in the blood of aborting cows may be determined by complement-fixation inhibition test of Nobuto, et al. (41).

*Salmonellosis:* *Salmonella dublin* was shown by Field (42) to be the cause of septicemia and enteritis in cattle of all ages. A high rate of abortion was noted in some outbreaks. In some instances abortion occurred following the acute disease, but in others abortion occurred when symptoms were minimal or absent. While there are no detailed accounts of the pathologic changes present in aborted fetuses, confirmation of salmonellosis is readily accomplished by isolation of the organism from fetal stomach contents. Uterine exudates will also yield cultures of salmonella.

III. ABORTION OF SPORADIC OCCURRENCE WITH LOW INCIDENCE WITHIN THE HERD

*Vibriosis:* Although vibriosis is usually characterized in the bovine by delayed conception and early fetal death, older fetuses are occasionally presented to the laboratory for diagnosis. When compared with the diseases already discussed, vibriosis is distinctive in that the incidence of abortion in the later portion of the gestation period is much lower. The presence of large numbers of the organism in fetal stomach contents may be determined by microscopic or cultural procedures.

*Infection with the avian tubercle bacillus:* The sporadic occurrence of bovine abortion due to the avian tubercle bacillus has been described by Burgesser and Schneider (43). Tubercle bacilli were demonstrated in the uteri and placentas of cows from herds known to be free from bovine tuberculosis. Intradermal testing with mammalian tuberculin elicited no reactions, while testing with avian tuberculin did produce reactions. Examination of smears from the placenta with Ziehl-Neelsen staining is recommended in cases of unexplained abortion.

*Mycotic infections:* The attempts by Austwick and Venn (44) to diagnose mycotic abortion in cattle on a routine basis have shown that the condition may be recognized regularly by the direct and cultural examination of aborted fetuses and placentas. The presence of hyphae in the fetal stomach content and the necrotic cotyledons, together with the isolation of the fungus in pure culture, were the chief criteria employed. Examination of 601 fetuses and 52 placentas resulted in the diagnosis of 41 cases of mycotic abortion. The fungi most frequently isolated were *Aspergillus fumigatus* (29 cases) and *Absidia ramosa* (four cases).

Twenty-five of the 2,000 aborted bovine fetuses examined by Van Ulsen (45) appeared to be infected with fungi. Nearly half the cases occurred after feeding moldy hay. Skin lesions were seen in only four cases and no illness of the dam was noted. Of the fungi isolated, 18 were aspergilus and five were absidia.
Previous to these reports, Bendixen and Plum (46) described the isolation of aspergillus from the placentas of 15 cows that had aborted. Furthermore, these workers demonstrated that this fungus would localize in the placenta and produce alterations that would lead to abortion.

Miscellaneous bacteria: On routine examination of fetal tissues and fluids such bacteria as streptococci, staphylococci and cornyebacteria are often isolated. The purity of the cultures and the sites of isolation will determine their etiological significance.

IV. MISCELLANEOUS AGENTS HAVING POSSIBLE SIGNIFICANCE IN BOVINE ABORTION

In addition to the above-mentioned agents, many additional etiologic factors, both infectious and non-infectious, must be considered in relation to bovine abortion. Trypanosomiasis due to Trypanosoma theileri was diagnosed by Levine, et al. (47), in a heifer that subsequently aborted. Dikmans, et al. (48), observed T. theileri in the stomach contents of an aborted fetus but did not isolate the organism in culture medium. Increases in titer to Leptospira pomona antigen, however, were noted in heifers which aborted during this same outbreak. Contamination of fetal bovine kidney tissue culture was reported by Lundholm, et al. (49). In this instance there is ample evidence that the trypanosome originated from an apparently normal bovine fetus obtained from a slaughterhouse.

Moll and Finlayson (50) noted abortion in two pregnant cows during the course of a respiratory disease that spread through a group of experimental animals. A viral agent recovered from one of the aborted fetuses was cytopathogenic for bovine kidney cells in vitro but its etiologic significance was not established. Subsequently Moll and Davis (51) reported the isolation and preliminary characterization of six cytopathogenic viruses from the feces of cattle in six herds with histories of respiratory disease and abortion.

In some herds cows aborted approximately one month after respiratory involvement. An aborted six to seven month-old fetus showed marked serosanguineous subcutaneous and intermuscular edema and considerable amounts of fluid in the serous cavities. Virus was isolated from ascitic, stomach and allantoic fluids. The etiologic relationship of the isolated viruses with the clinical conditions mentioned was not established.

An epizootic of mucosal disease in North Dakota in 1956 was characterized by extensive calf-crop losses according to Shipper and Eveleth (52). In some instances, the entire calf crop was lost or born dead. In some herds, calves appeared normal at birth but died 18 to 96 hours later, while in other herds abortions were frequent. Histories of herds incurring extensive calf losses due to mucosal disease gave no indication of this entity having occurred or existing in any of the older animals except in one instance. The fetal pathology consisted of necrosis of nasal turbinates, acute pharyngitis, petechiae and ecchymoses of the trachea, punched out ulcers of the esophagus, petechiae of gastrointestinal mucosal, and no gross hepatic pathology. All attempts to transmit the disease were unsuccessful.
DIFFERENTIAL DIAGNOSIS OF BOVINE ABORTION

Rift Valley fever and Wesselsbron viruses are widespread in South Africa and are recognized as causes of abortion in sheep and cattle (53). Although antigenically distinct, both viruses produce disease which is similar clinically and epizootiologically.

The capacity of modified live virus vaccines to produce fetal malformations and abortion has been shown to occur during the immunization of swine against hog cholera (54) and sheep against bluetongue (55). The increasing use of modified live virus vaccines to combat bovine diseases such as infectious bovine rhinotracheitis, may result in abortion, particularly if immunization is instituted during the early portion of gestation.

In herds where abortion remains unexplained after the completion of routine laboratory procedures, a search for metabolic or genetic factors must be instituted. The development of erythrocyte antibodies as a cause of abortion in cattle has been suggested by Laing and Blakemore (56). Several positive sera were found in cows tested by the Rh agglutination technic against red cells of bulls to which they had been mated. Abortion ensued when such cows were transfused with whole blood obtained from certain bulls. The fetal blood had many erythroblasts and in one case all red cells were lysed.

The abortion of fetuses exhibiting evidence of dwarfism was noted by Bolin, et al. (57), in herds where infectious causes of abortion were eliminated. Fetuses submitted for examination exhibited tissue edema, hydrothorax, ascites, spheroid heart, closed occipital condyles, and abnormal lumbar vertebrae. These observations suggest that cardiac insufficiency is a cause of abortion in herds where dwarfism is a problem. One instance was cited in which a cow aborted in two succeeding years.

The ingestion of poisonous plants is often suggested as a possible cause of abortion, particularly by the owner of the animals involved. The instances in which poisonous plants are confirmed as the causative factor are usually characterized by the development of severe symptoms in the dam and occasionally death. These are the characteristics of perennial broomweed (58) and Macrocarpa (59) poisoning.

Deformed or “acorn calves” are common in the oak belt of the Sierra Nevada foothills during dry years when animals are confined on poor feed (60). The deformities may be so grave that the fetus is born dead, or would die quickly if unaided. Acorn calves are so-called because of a rather general impression that they result from the eating of too many acorns by the dam during gestation. While this is not the case, excessive acorns in the diet may prevent the formation or utilization of some essential food element and thus aid in producing deformed or dead calves. A similar condition may occur when vitamin A deficient cattle consumes excessive quantities of pine needles.

SUMMARY

A system of classification for bovine abortion is proposed which places emphasis on the clinical and epizootiological aspects of the diseases involved. Abortion diseases having epizootic occurrence are defined as those having
widespread distribution in a geographical area during a definite time interval and with heavy losses within the affected herds. Included in this category are brucellosis, leptospirosis, epizootic bovine viral abortion and the "Lowland Abortion Syndrome."

Abortion diseases with sporadic occurrence, but high incidence within the herd, included listeriosis, toxoplasmosis and salmonellosis. Although recognition of toxoplasmosis in cattle has been very limited, it is included in this category because of the widespread nature of the disease in New Zealand sheep.

Diseases with sporadic occurrence and low incidence within the herd include avian tubercle bacillus infection, mycotic infections and vibriosis. Even though a high incidence of infertility or early abortion may occur in vibriosis, it was classified here because of the low percentage of abortions which occur during the last trimester of pregnancy.

In discussing miscellaneous agents having possible significance in bovine abortion in the United States, both infectious and non-infectious factors were considered. Those receiving mention were as follows: Trypanosoma theileri, cytopathogenic enteroviruses, mucosal disease, Rift Valley fever virus, Wesselsbron virus, modified live virus vaccines, development of erythrocyte antibodies, bovine dwarfism, poisonous plants and deficiency diseases.

### TABLE 1

Serologic Results Obtained in Two California Range Beef Herds Tested With *Leptospira pomona* antigen

<table>
<thead>
<tr>
<th>Herd</th>
<th>Total Cows*</th>
<th>Neg.</th>
<th>1:10</th>
<th>1:100</th>
<th>1:1,000</th>
<th>1:10,000</th>
<th>Average Titer</th>
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<tbody>
<tr>
<td>V</td>
<td>Non-parturient</td>
<td>80</td>
<td>29†</td>
<td>10</td>
<td>24</td>
<td>16</td>
<td>1:356</td>
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<td></td>
<td>Parturient</td>
<td>407</td>
<td>388</td>
<td>15</td>
<td>3</td>
<td>0</td>
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<tr>
<td>S</td>
<td>Non-parturient</td>
<td>98</td>
<td>58</td>
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<tr>
<td></td>
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<td>676</td>
<td>637</td>
<td>28</td>
<td>11</td>
<td>0</td>
<td>1:2</td>
</tr>
</tbody>
</table>

* Bled at the end of the calving season.  
† Agglutination test; killed antigen.

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DIFFERENTIAL DIAGNOSIS OF BOVINE ABORTION


DIFFERENTIAL DIAGNOSTIC METHODS FOR ABORTION IN SWINE

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Abortions in swine are associated with both noninfectious conditions and a number of infectious agents. Time does not permit a complete discussion of each of these factors and agents so our objective in this discussion will be limited to diagnostic procedures which are useful in the investigation for causes of any case of abortion in swine.

The two most common causes of abortion in swine are probably brucellosis and leptospirosis. In each of these infections, the routine and fastest method of demonstrating the presence or absence of the infectious agent is by means of a serological test with blood from the dam. The agglutination test for brucellosis is a dependable indicator, however, for conclusive proof, efforts should be made in each case to cultivate the causative bacterium from the specimen fetus. If cultural methods prove successful, differential brucella typing methods may be used if desired.

In leptospirosis, positive agglutination test results are subject to questionable interpretation unless they are supported by good clinical evidence or a very high titer. Bacteriological methods for cultivation and identification of the causative organism may be used, however, this procedure is time consuming and the percentage of successful isolations is rather low. Histopathological examination of silver stained kidney sections from the fetus are much more useful in swine than in cattle. Occasionally, dark field examination of urine from the dam or of urine and kidney scrapings from the fetus may provide evidence for a tentative diagnosis.

For convenience, other bacterial organisms associated with swine abortions can be discussed as a group. It is composed of erysipelothrix, listeria, streptococcus, and corynebacterium organisms. The usable diagnostic procedure for each of these agents consists of bacteriological methods to culture and identify any of these organisms which may be present in the aborted fetus. In addition to recovery of any of these bacteria, it is necessary to eliminate other causes by means of history, serological tests, and any other applicable procedures. The occurrence of any of these bacteria associated with swine abortions is quite erratic and not common. In a very few cases observed in which erysipelothrix was recovered, the abortion occurred at very close to full term. Some of the litter were dead at birth, others survived for a few hours and edema was quite noticeable. The over-all picture in these cases was quite similar to that of leptospirosis. However, no evidence of leptospirosis could be developed either from the fetuses or the sows. Streptococcus and corynebacterium organisms are very common in swine and abortions may

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occur in droves in which the level of infection with these bacteria becomes quite high. These droves are usually those in which management and sanitation is below average and consequently the general health level of the drove is below normal. Almost invariably, other conditions such as umbilical infection, enteric conditions, atrophic rhinitis, and pneumonia can be found in the adult animals of these droves.

Bacteriological examination of aborted swine fetuses occasionally results in the recovery of a practically pure culture of mycotic agents. The encounters in the Iowa laboratory with this infectious agent involved either aspergillus or penicillium species and the aspergillus has also been recovered from bovine abortions. In these cases it is always necessary to eliminate, as far as possible, any other causes of abortion, either infectious or noninfectious. Histopathological examination, using a fungus stain such as Gridley's, sometimes gives valuable support to the findings by other methods.

A small group of virus infections should also be considered among the infectious causes of swine abortions. These include cholera, vesicular exanthema, and foot-and-mouth disease. The two vesicular diseases have been eradicated in this country, but the possibility of a recurrence should always be kept in mind. A diagnosis of these diseases would be made on the adult swine on the basis of history, gross lesions, serological methods, and animal inoculation tests are always advisable. Cholera outbreaks are sometimes accompanied by abortions. Mortality of the adult animals usually attracts the attention of the owner more than the abortions so the abortions are sometimes overlooked in the history which is related in efforts to learn the cause of trouble in the adult. Practical methods for the diagnosis of cholera will depend upon a history, clinical evidence, and post-mortem examination of the adults rather than upon evidence secured from a specimen fetus alone.

On rare occasions, abortions due to stilbestrol may be encountered. In these instances, it is necessary that an accurate history of availability of stilbestrol for ingestion by the sows be secured. They also require the elimination of other causes associated with swine abortions. Very sensitive biological assay methods for stilbestrol are available if needed.

Nutritional causes of swine abortion are not rare but they are often quite complicated and difficult to establish. In any case, the infectious causes should always be eliminated before considering nutritional factors. Such consideration will be concerned primarily with adult animals and must take into account the environment, management practices, their nutritional requirements and the ration they have available, and concurrent diseases and parasitism. In a few instances, specific deficiencies may produce recognizable lesions of the fetus. For example, iodine deficiency usually results in edema and hairlessness. Vitamin A deficiency may produce palatognathism, microphthalmia, and anophthalmia. Most deficiencies, however, are usually multiple in character and gross lesions cannot be depended upon to complete a diagnosis.
From this comparatively large list of infectious agents and noninfectious factors associated with swine abortions, it is apparent that the diagnostician must be prepared to cover several wide fields of investigation on each case he may examine. He should have a very good history of any clinical symptoms which may accompany the abortions. The history should also include as much as possible of the environment, feeding and management practices, and association with other species of animals. A rather wide variety of bacteriological media should be available for use and cultural methods should include the use of various temperature and atmospheric conditions. He must also be prepared to use specially stained histopathological sections, animal inoculation tests, and biological assays. Consideration of the adult animals should never be neglected since their serological status and clinical history are often vital elements in establishment of a diagnosis when abortion is involved.
### Differential Diagnostic Methods for Abortion in Swine

<table>
<thead>
<tr>
<th>Cause</th>
<th>Diagnostic Procedure</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria</td>
<td>Serology. Dams blood.</td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Bact. methods. Culture and identify causative agent.</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Bact. methods. Eliminate other infectious causes.</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>Bact. methods. Eliminate other infectious causes.</td>
<td></td>
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<tr>
<td>Virus Infections</td>
<td>Diagnose disease in adult animals. Cholera—clinical history and gross lesions</td>
<td>Animal inoculation tests.</td>
</tr>
<tr>
<td>Cholera</td>
<td>VE Rare. History, gross lesions, serology. Animal inoculation tests.</td>
<td></td>
</tr>
<tr>
<td>Estrogens</td>
<td>History of availability for ingestion by sows. Elimination of other causes.</td>
<td>Biological assay.</td>
</tr>
<tr>
<td>(Stilbestrol)</td>
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</tbody>
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LABORATORY CONFIRMATION OF EQUINE ENCEPHALOMYELITIS

ROBERT J. BYRNE*

College Park, Maryland

INTRODUCTION

The early recognition and laboratory confirmation of equine encephalitis is of vital importance to Livestock Sanitary Service officials. The information is essential in insuring prompt control measures for the suppression of the disease in domestic animals. Laboratory confirmation of early cases in animals also serves to alert public health officials of the potential danger to human beings.

For the proper selection, and testing of material for laboratory examination and a better understanding of results, one should first be acquainted with the course of infection in animals. See Figure 1. Following exposure to eastern equine encephalitis (EEE) virus in nature, or in the laboratory, horses usually circulate virus in their blood for two to three days. This

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period of viremia may or may not be accompanied by fever. The level of viremia in horses, i.e., an LD$_{50}$ of between $10^{-2}$ and $10^{-3.5}$, is not as high as that observed in small passerine birds, but can be of sufficient magnitude to infect low threshold mosquitoes such as the common salt marsh variety, *Aedes sollicitans*. In nature, this phase of infection would probably be missed and it is doubtful that blood specimens would be obtained for laboratory examination at this time. Further, it is quite probable that greater than half of all EEE infections in horses are subclinical, and never develop clinically recognizable disease. As one would expect, no circulating antibody of any type is demonstrable during the stage of viremia.

After the cessation of viremia, there is usually an abatement of fever, if it had been present, and the animal may appear normal. In those horses which are destined to develop clinical encephalitis, there is a second wave of fever, depression and first signs of incoordination. From this point, the severity of the disease increases rapidly and at least in the case of EEE, horses may die in two to four days following onset of clinical signs. The mortality in EEE affected horses is greater than 90 percent, although in the case of western equine encephalitis (WEE), it is lower. Antibody development during the stage of clinical disease is very rapid and at the time of death, horses and ponies affected with EEE will have a significant level of both hemagglutination-inhibition (HI) and neutralizing antibodies.

In those animals which die in two to three days after first showing central nervous system (CNS) signs, virus can usually be isolated from the brain, providing that proper care is taken in handling of material for examination. When the disease is prolonged, and death finally ensues, chances for virus isolation from the brain are greatly diminished.

Animals which recover from the disease or experience only subclinical infections, are left with a high level of serum HI and neutralizing antibodies (1). Complement-fixing (CF) antibodies, when they do appear, are detected three to six weeks after exposure to EEE virus and are of short duration (2, 3). Thus, their value in establishing a diagnosis of EEE is limited by the high mortality in the disease, inconsistent appearance of CF antibody and the relatively poor chances of obtaining properly matched serums.

Since we have worked less with WEE virus in horses than EEE, and there is little information available on viremia and antibody response of horses to WEE, a comparison is somewhat difficult. However, it is known that viremia in horses infected with WEE is so inconsistent and fleeting as to virtually exclude the horses from any role in the transmission of the disease (4). In the horses we have injected with WEE virus, there was an antibody response as measured by HI and neutralization tests (3). The HI antibody response was less marked and of shorter duration than that observed in EEE infected horses. This might be a reflection of the differences in the level of viremia seen in the two infections. Unfortunately, we have had no opportunity to examine serum from natural cases of WEE, but would welcome the opportunity to do so.
Regarding detailed laboratory techniques involved in the study of equine encephalitis, no review is in order since there is ample literature on the subject. The main categories to be considered in this presentation are isolation methods and serological tests.

The isolation of equine encephalitis virus from the brains of horses dying of the disease is not a problem requiring very exacting or tedious methods. The brain should be removed from the animal as soon as possible after death and kept refrigerated until brought to the laboratory. At this point, representative portions should be taken and placed in formalin for later sectioning and histological examination. Other pieces are removed and ground into a 20 percent emulsion using a 50 percent heat inactivated normal rabbit serum (NRS)—physiological saline diluent. Laboratory animals, embryonated eggs and tissue cultures may be inoculated from this emulsion after it has first been subjected to low-speed centrifugation and dilute to $10^{-1}$. Addition of antibiotics to material for inoculation is usually indicated. Remaining portions of the brain and unspun 20 percent emulsions are quickly frozen and stored at $-50$ to $-70\degree C$. It should be pointed out that the best portions of brain for isolation attempts are the basal ganglia, thalamus, and parietal and frontal cortex of the cerebrum (2).

Either suckling or recently weaned white mice are injected intracranially (IC) with the $10^{-1}$ suspension of horse brain, the dose varying from 0.01 to 0.03 ml. Guinea pigs or rabbits may also be injected by this route, and a dose of 0.1 may be used. The wet or day-old chick is highly susceptible to both EEE and WEE viruses (5, 6) and is our preferred test animal. Chicks are inoculated subcutaneously with 0.1 ml. of tissue suspension and will develop signs of disease 18-24 hours later if EEE or WEE viruses are present.

In general, young mice inoculated IC with positive brain material will develop CNS signs in 48 to 76 hours. The incubation period may be somewhat longer in older mice and in guinea pigs and rabbits. The factors influencing results of isolation attempts include virus content of the original tissue suspension, age of the test animals and, possibly, variations in virus strains. Also to be considered are the effects of interference from dead virus particles and the presence of serum neutralizing antibodies which appear shortly before death.

Our preference for the baby chick is based on the fact that we have made virus isolations from horse brains that we would have missed if we had relied on inoculation of adult mice by IC route. We have not carried out any critical studies on comparison of day-old chicks and suckling mice as test animals.

Identification of isolates is made by cross neutralization studies in mice employing known strains of EEE and WEE viruses and antiserums. Preliminary typing can be made by injecting the suspension of the original virus-positive brain or low-mouse brain passage material into two to three week old chickens (6, 7). Birds are bled prior to inoculation and 10 to 14 days later. Chickens of this age are resistant to disease, but respond rather
promptly and specifically to EEE and WEE virus as measured by the serum HI test.

In short, isolation, per se, of equine encephalitis virus from properly selected and treated material, offers no special challenge to the average laboratory. The most serious problem that arises, is that involving the health of individuals working with virus isolates. No commercial EEE or WEE vaccines for human use are available at present. Therefore, I would not recommend extensive passage work or antigen preparation in any laboratory not prepared to work with the equine encephalitis viruses.

The use of serological methods in diagnosing equine encephalitis in horses has been neglected, despite availability of a variety of well-known tests. In brief, in vitro neutralization tests are conducted on unheated serum, using mice as indicator animals. Undiluted portions of serum are combined with appropriate logarithmic dilutions of virus and, after a two-hour incubation period at 37° C., indicator animals are inoculated IC with .03 ml. doses of the mixtures. Results of observations are tabulated after 10 days and expressed as the neutralization index. A better quantitative estimate of serum neutralization antibody may be obtained by using serial 10-fold dilutions of serum in combination with fixed quantities of virus, usually in the neighborhood of 100 mouse LD₅₀'s. Using the latter method, it is possible to demonstrate a true increase in antibody titer between paired serums.

Commercial antigens are available for EEE and WEE complement-fixation tests although a problem in establishing the optimum number of antigen units arises unless standard reference serums are on hand. Descriptions of the CF test for EEE and WEE are given in the references (8, 9, 10).

The HI test for EEE was first described by Casals (11). Subsequent modifications and descriptions of its application for both EEE and WEE are given (1, 12, 13). In our experience, borate saline antigens prepared from EEE and WEE infected mouse brains are sensitive and stable. Moreover, they appear to lose their infectivity over an extended period and could be safely used in laboratories which routinely perform HI tests. Serums must be extracted with acetone prior to testing to remove any non-specific inhibitors.

In laboratories equipped to do histopathological examinations, valuable supporting information may be obtained from such studies. In some instances, particularly those in which no virus isolation is made from the brain, the microscopic lesions are the only clue to the cause of the disease. Unfortunately, the lesions due to the various arthropod-borne encephalitis viruses are so similar as to preclude a differential diagnosis, within the group, on this basis. In brief, the chief microscopic lesions in the brain are perivascular cuffing, and neuronal degeneration. There are no inclusion bodies associated with the disease.

For a comparison of methods in our laboratory to confirm equine encephalitis, refer to the table. Of further interest is the fact that, using a combination of the methods shown in this table, we were able to confirm 13 out of 18 cases of equine encephalitis in Maryland in August and September of 1960.
COMPARISON OF METHODS USED FOR LABORATORY CONFIRMATION OF EQUINE ENCEPHALITIS

<table>
<thead>
<tr>
<th>Case</th>
<th>Place of Occurrence</th>
<th>Year</th>
<th>Serological Tests</th>
<th>Examination of Brain</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HI Titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EEE</td>
<td>WEE</td>
</tr>
<tr>
<td>1</td>
<td>Reisterstown, Md.</td>
<td>1956</td>
<td>640</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Cambridge, Md.</td>
<td>1958</td>
<td>2560</td>
<td>&lt;10</td>
</tr>
<tr>
<td>3</td>
<td>Berwyn, Pa.</td>
<td>1959</td>
<td>1280</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4a</td>
<td>Estelle Manor, N. J.</td>
<td>1959</td>
<td>2560</td>
<td>10</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td>160</td>
<td>10</td>
</tr>
<tr>
<td>5a</td>
<td>St. Mary's City, Md.</td>
<td>1960</td>
<td>10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td>160</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* All isolates were typed as EEE.
† b: Indicates second serum sample from same horse.

Horse four made a complete recovery. Serum “a” was taken during acute phase of disease; serum “b” was taken six months later.

Horse five died. Serum “a” was taken at onset of C. N. S. signs; serum “b” was taken 1-2 days later.

DISCUSSION AND SUMMARY

The prompt confirmation of equine encephalitis in a state or community is an important animal and human health consideration. For this purpose, every effort should be made to obtain blood specimens from animals showing signs of equine encephalitis and the brains from those which die of the disease. As illustrated in the figure, a serum obtained from an animal in the terminal stage of EEE, invariably has a high HI titer. In the event serum was obtained at the very first sign of disease and again at the time of death, a rise in titer can be shown with the HI test, thus establishing serological proof of infection. There is little or no crossing between WEE and EEE viruses as measured by HI (14).

Final definitive confirmation of EEE and WEE is made through isolation and typing of the virus. A preliminary typing may be made by inoculating chickens with virus suspension and demonstrating an HI antibody response in the injected birds.

REFERENCES


Infectious synovitis (IS) was first recognized as a separate disease entity in 1954 in West Virginia (1) and Texas (2). It was soon recognized in all major broiler producing areas in United States.

The cause of the disease was thought to be a large particle virus or small bacterium (3, 4). However, it was identified as a *Mycoplasma* by Lecce (5) who could culture the organisms on swine serum PPLO agar plates in the presence of a staphlococcus streak and 10 percent carbon dioxide. This finding was confirmed by Chalquest and Fabricant (6) who was able to grow the organism in PPLO broth media containing 10 percent swine serum, 0.1 percent cysteine (free base) and 0.1 percent diphosphopyridine nucleotide (DPN) plus inhibitors. The PPLO isolated from IS of chickens is not antigenically related to *Mycoplasma gallisepticum*, the cause of chronic respiratory disease.

These findings help clarify the transmission of synovitis since PPLO are commonly egg transmitted. Many investigators presented evidence that the IS was transmitted through the egg (3, 7, 8). The rate of egg transmission was generally low but with virulent strains the disease spread by contact in 25 days or more so that a morbidity of 20 percent was common. Occasionally up to 75 percent of a flock showed infection with a 10 percent mortality. Also, condemnations were high in dressed birds from affected flocks.

Benton and Cover (9) found in intravenous inoculated birds, that the agent reached its maximum titer in the blood in 72 to 96 hours, and persisted until the 15th day but did not persist for 52 days. The organism was found in nearly all tissues, however, it appeared to be more concentrated in the synovial fluid. In field cases the hock joint and the bursal synovial membrane are the areas most commonly affected. Chickens are susceptible by nearly all routes of inoculation, however, the joint or synovial membrane route of inoculation is most apt to result in infection. This is especially true when the virulence of the agent is reduced or when a small number of infective particles are present.

The signs of IS are lame, droopy, emaciated birds with pale combs. Breast blisters and swellings about the joints are present. On autopsy, the spleen, kidneys and liver are enlarged and the latter is frequently mottled. The enlarged joints contain a creamy viscous fluid in the early stages of the disease. In later stages a caseous exudate may be present or an orange discoloration of the joints may occur or both. Breast blisters occur which also contain pus or a caseous exudate. May-Greunwald and Giemsa stained smears of the creamy exudate contain a large number of mononucleated cells.

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DIAGNOSIS OF INFECTIOUS SYNOVITIS

This in contrast to a staphlococcal arthritis where large numbers of heterophils are found and staphylococci are present.

In turkeys the infection is less acute and all that may be noted is lameness. If these birds are picked up, warm fluctuating swellings may be noted about the hocks. Morbidity is usually low and mortality usually results from cannibalism. In chickens the infection is usually noticed at six to 10 weeks of age whereas in turkeys, the infection appears later. In turkeys a staphlococcus infection is more common, but the reverse is true in chickens.

The microscopic lesions of IS have been described by Sevoian et al. (10). In acute cases the liver shows a perivascular, periportal and interparenchymal cellular hyperplasia of the reticular cells of the reticulo-endothelial system. Similar hyperplasia is found in the connective tissue framework of the heart, gizzard, and the interlobular septa of the lungs. Hyperplasia of reticular and/or lymphocytic cells decreases the sinusoidal areas of the spleen. A granulocytic hyperplasia of the bone marrow occurs.

Average determinations of the blood components of 31 experimentally inoculated birds were as follows: erythrocytes, 1,680,000 per mm. (3), leucocytes, 80,810 per mm. (3), hemoglobin 6.5 gm. per 100 ml. of blood. Differential count gave the following percentages: lymphocytes, 41.9; heterophils 31.9; monocytes 19.3; eosinophils 0.23; basophils 1.1; immature leucocytes 6.1. The severity of the blood changes generally correspond with the severity of the disease.

The diagnosis of infectious synovitis, in typical uncomplicated cases, can usually be made on the basis of signs and lesions. The histologic and hematologic changes are also a help in reaching a diagnosis. However, in many flocks the disease is complicated with chronic respiratory disease or other bacterial infections. Numerous organisms which may gain access to the synovial fluid and elicit some of the signs associated with IS are: Staphylococcus, Salmonella, Pasteurella, Mycoplasma, Escherichia, Erysipelothrix. The fowl pox virus will also grow and cause swellings in the joints of birds.

To confirm a diagnosis of infectious synovitis, fluid should be aseptically collected from the joint. The fluid may be diluted one to 10 with nutrient broth and inoculated in 0.25 ml. amounts into the yolk sac of five- to seven-day-old embryonating chicken eggs or the foot pad of two- to four-week-old chickens. Thioglycollate media, nutrient agar and PPLO broth are inoculated to check for bacteria. We use Difco’s PPLO broth without crystal violet with the following percentage of enrichments; horse or swine serum 10 and yeast hydrolysate 10. The latter is prepared by placing one pound of bakers yeast in 500 ml. of distilled water. This is incubated at 56° C. for five days, centrifuged and the supernatant filtered through a Sietz EK filter. After three passages in broth the culture is plated on PPLO agar that contains the above enrichments.

If the infectious synovitis agent was present a swelling should develop in the foot pad of the inoculated chick in four to 10 days. Embryo mortality should occur in five to 15 days. In some cases, the embryo may survive to hatching time. The embryos that die before the formation of feathers are
markedly hemorrhagic, the spleen is enlarged and the liver is enlarged or mottled or both. Embryos which die after the feathers are formed, usually have enlarged livers and kidneys which may or may not be hemorrhagic. The spleen is usually enlarged and it frequently contains necrotic foci. If the above characteristics occur in chickens and embryos, and no bacteria or PPLO are recovered, one is justified in making a positive diagnosis of infectious synovitis.

If further differentiation is desirable the material harvested from infected embryos or joints can be cultured in PPLO broth (Difco), without crystal violet, that contains 0.1 percent DPN and 0.1 percent cysteine plus 10 percent swine serum. The broth can be incubated in regular atmosphere but agar plates with the same enrichments should be placed in a candle jar. Since the DPN is rather unstable, a one percent solution is made and kept frozen. Just before use, one ml. of the solution is added to 10 ml. broth. Transfers should be made every three days.

**SUMMARY**

Infectious synovitis can usually be diagnosed on the basis of signs and lesions. When the flock history suggests that complicating diseases are present or when only a few birds are involved, fluid from infected joints should be inoculated into chickens, embryos and cultured in bacteriological media to confirm the diagnosis.

**REFERENCES**

THE SPECIFIC PATHOGEN FREE PROGRAM AND ITS INFLUENCE ON VETERINARY DIAGNOSTIC LABORATORIES


Lincoln, Nebraska

Modern methods of livestock production are designed to produce more livestock in less time, with less labor and in a smaller area. Confinement technics are being used more in poultry and swine production than with any other livestock. An increase in the density of population also increases the danger of disease transmission by contact. To reduce the danger of this situation, disease prevention must be an essential part of swine or other livestock enterprise. It is recognized that healthy livestock can make more efficient use of available feed, and in turn make maximum growth and reproduce to the full extent of their genetic ability.

The members of the veterinary profession and American livestock industry have employed many technics in disease control and eradication programs. These include embargo to prevent the introduction of disease into the nation or quarantine to confine disease to an area or a specific premises. Elimination of diseased animals by slaughter as in foot-and-mouth disease or by test and slaughter in the tuberculosis and brucellosis eradication programs are two other technics. Control of many diseases has been accomplished by the use of vaccination procedures. The measures mentioned have been directed primarily toward the control of acute infectious and contagious diseases. Until recently very little had been done to eliminate those chronic diseases of swine which produce low mortality, but seriously effect growth rate and efficiency of feed conversion. Part of this inertia may have been due to lack of knowledge concerning etiology as in infectious atrophic rhinitis or satisfactory methods for detecting such diseases as virus pneumonia of pigs in the living animal. Until this information is available it will be difficult to determine the exact time or avenue of infection of these diseases. Current information suggests transmission from infected sows to their offspring during the suckling period.

A method for the control and possible eventual eradication of some of the chronic swine diseases is available in the Specific Pathogen Free (SPF) Swine Program. This program makes use of the technics employed in other disease control programs as well as application of modern aseptic procedures. These include isolation of pigs taken by hysterectomy to prevent exposure to diseases from other swine or human sources. This is accomplished with aseptic methods not unlike those used for the protection of human infants. Elimina-

* Department of Veterinary Science, College of Agriculture, University of Nebraska, Lincoln, Nebraska.
tion of infected sows by slaughter and removal of other swine before re-
population might be compared to the previously mentioned foot-and-mouth 
eradication measures. When an infected sow is eliminated under this program, 
her blood line is perpetuated through the pigs and the carcass is salvaged for 
food.

The SPF program offers a means of eliminating certain chronic conditions 
or diseases transmitted by contact. No one associated with the program feels 
that it will be the means of ending all swine diseases. Pathogens which live 
in the soil, such as *Erysipelothrix rhusiopathiae* or parasite ova, cannot be 
eliminated under this program. The individuals responsible for maintaining 
the health of an SPF herd are the practicing veterinarian and the owner. 
The practitioner will find it advantageous to submit specimens to the diag-
nostic laboratory for confirmation of his clinical diagnosis. Progressive hog 
men raising SPF hogs will find it essential to plan with their veterinarians a 
program of preventive medicine this will include vaccination for diseases 
such as hog cholera, erysipelas, and leptospirosis with parasite control being 
an additional component.

We will not take time to discuss the technic or equipment for delivering the 
pigs by hysterectomy or their incubation and care. These aspects of the SPF 
program have been previously described in the literature (1, 2, 3, 4). Veteri-
nary diagnosticians should familiarize themselves with the technic and prin-
ciples involved. It is such knowledge that will permit the diagnostician to be 
of service in this program. The diagnostic laboratory can play an active role 
in at least three phases of the SPF program.

**PHASE ONE:**

The Diagnostician can be of service to hogmen when pigs carrying virus 
pneumonia or atrophic rhinitis are examined in the laboratory. Familiarity 
with the SPF program will permit the diagnostician to suggest to and discuss 
with the producer a means of eliminating these diseases from his herd. It is 
not unusual for owners to recall having read about the repopulation program 
in their farm journals. When they see the signs of these chronic diseases in 
their own animals and the SPF program is offered as a solution there is a 
stimulation of interest. Thorough knowledge of the SPF program will enable 
the diagnostician to act as an impartial source of information at a time when 
a particular herd is experiencing difficulty. Should it be the preference of 
the diagnostician, he may refer the swine producer to a veterinarian devoting 
his practice to SPF hogs.

**PHASE TWO:**

Laboratories set up to produce primary SPF pigs are licensed by the 
University of Minnesota. Patents on the equipment and procedure are held 
by that institution. The professional training required to establish and 
operate such a laboratory falls in the province of the veterinarian. Recog-
nizing this, the University of Minnesota requires that a laboratory have a 
veterinarian to direct the work before a license is granted. Veterinarians
operating laboratories producing primary SPF pigs will probably restrict their practice to this phase. Veterinarians producing SPF pigs will solicit the help of Diagnostic Laboratories if and when they run into unexplainable losses in their incubators and brooders.

It seems probable that veterinarians engaged in this endeavor would follow the recommended procedures in detail, this is true as far as their particular laboratory will permit. However, in establishing any enterprise, there are certain things that must be learned by experience. An example of this is the quantity of milk to place in a feeding pan. To the uninitiated this may sound like a simple thing. A suggested amount of milk for a day old pig is about one ounce per feeding. The important thing is that it must be spread out in a shallow pan so that the nostrils are not submerged while the animal drinks. What happens if the nostrils are submerged? The result is inhalation of milk with possible drowning or the development of mechanical pneumonia and eventual death. This is a simple item, but to the pig just learning to drink it is very important. It grows in importance if more than one type of feeding pan is being employed in a laboratory. Each primary laboratory must, in addition to the veterinarian, have caretakers to do most of the actual feeding and cleaning of equipment. Most people engaged for such work have no background in aseptic technics and are inclined to take short cuts or omit precautions set up to prevent spread of disease between animals or from humans to the young pigs. It must be remembered that primary SPF pigs are colostrum deprived and antibody devoid animals. Because of this they are susceptible to human as well as porcine infections. Dr. A. O. Betts of Cambridge, England, mentioned at the July 1960 Repopulation Seminar held in Lincoln, Nebraska, that one of their biggest problems has been the prevention of disease spread from human caretakers to the young pigs. He cited instances where a young woman caretaker contracted a respiratory infection and in spite of precautions the infection appeared in the pigs she was feeding. Diarrheas have been reported in incubator pigs following the same condition in their caretakers.

Such infections offer a challenge to the Diagnostic Laboratory to isolate and identify the human pathogens from these pigs.

Primary pigs, those delivered by hysterectomy, are usually removed from the brooders at three to four weeks of age and placed on farms. Transfer from a well-controlled environment to more natural surrounding requires adjustment by the pig. It also presents a time when contact is made with bacteria and other agents on the farm. Should the adjustment be unsatisfactory the Diagnostic Laboratory will be asked to determine the cause.

**Phase Three:**

A bigger role will be played by the Diagnostic Laboratory in solving problems in the secondary herds. These herds will be composed of SPF hogs which are the result of natural farrowing. The program of disease prevention as set up by the veterinarian and the skill and diligence of the owner in keeping his herd isolated and free from outside infections will be
reflected in the problems he has for his veterinarian and by referral to the Diagnostic Laboratory.

Removal of chronic infection combined with improved sanitation and management has increased the growth rate in SPF pigs. More rapid growth has, of course, influenced the nutrient requirements of these individuals. It is not unlikely that some herds will have nutritional problems, not recognized until the animal is sent to the Diagnostic Laboratory.

One producer used the old axiom when breeding his gilts, "If they are big enough, they are old enough." As a result his gilts farrowed just past eight months of age and produced small, unsatisfactory litters. It is essential that these animals be allowed to mature before being used in the breeding herd.

Hogs in secondary herds are, for all intents and purposes, normal hogs as far as susceptibility is concerned. They are susceptible to those pathogens to which they have not been exposed or immunized against. Because they are SPF animals they are no more susceptible than an animal produced under some other system. SPF hogs may actually be less susceptible than others from the standpoint that they are not carrying a subclinical chronic condition which may reduce their resistance to a newly introduced pathogen. Animals from secondary herds, when examined at the Diagnostic Laboratory, will usually be suffering from only one condition rather than an acute infection complicated by the presence of other pathologic conditions.

In closing, Veterinary Laboratory Diagnosticians recognize that the activities of Diagnostic Laboratories have been influenced by the development of new disease control methods. Disease eradication programs combined with the discovery and use of effective new immunizing agents all reduce the incidence of specific diseases. Hog cholera and swine erysipelas are two excellent examples.

The Specific Pathogen Free pig program will have a similar effect in regard to many of the chronic swine diseases. This will allow the Diagnostic Laboratories to direct their activities toward cooperation with the veterinary profession and livestock industry in the control and eradication of still other diseases.

BIBLIOGRAPHY

At this meeting and at similar meetings, we hear of new virus diseases and of new methods of culture, identification and control of viruses. Almost every state experiment station is engaged in some phase of virological research. Few would deny its importance to the veterinary profession.

Although the research in virology is widespread in the United States, only a few methods for the diagnosis of virus diseases have become routine in laboratories that do the major share of veterinary and medical diagnostic work. A significant lag exists between development of methods and their utilization.

The subject of this paper concerns ways of shortening the lag—of encouraging the use of virological methods—and of doing this without the need for making major staff additions or considerable increase in equipment.

Any laboratory in which bacteriological diagnostic work is done can also handle diagnosis of many of the viral diseases. The basic methods are similar: the isolation of the etiological organism by animal inoculation; the demonstration of serological specificity and the demonstration of pathological specificity.

The essential equipment used for the diagnosis of bacteriological and virological infections is the same. I would include seven items of equipment:

1. cages
2. an autoclave
3. a standard centrifuge
4. a standard microscope
5. a refrigerator, and, if possible, a freezer
6. serological water baths
7. a bacteriological or egg incubator

The latter is needed if extensive virological work is to be undertaken. One does not need an expensive ultracentrifuge, an electron microscope, or lyophylizer to do virological diagnostic work.

On the other hand, some of the inexpensive materials used in virological diagnosis are not regularly available in a bacteriological laboratory. It is the absence of these materials that is often the limiting factor. First, it is necessary to have a suitable supply of animals or animal tissues in which viruses can be isolated and in which neutralization tests can be performed. Because of the cost, few laboratories, other than the large research institutes maintain animal breeding colonies. A solution for even the smallest laboratory is to locate a source of supply from which animals or tissues may be obtained within one or two days. Our experience in Madison and in a field laboratory
in Waycross, Georgia, suggests the following. Rabbits are bought locally at 60 and 70 cents a pound for about $3.00 for a five-pound rabbit. Guinea pigs are purchased at approximately $2.00 each, although good animals are difficult to obtain. Mice are obtained locally or from a national supplier such as Carworth Farms, New City, New York. Individual weaned mice cost about 30 cents each, and pregnant females are priced at $1.15 each. Chickens are obtained year around as baby chicks at 10 to 15 cents each, or as adults at $1.50 each. In Madison we pay market rates, 50 to 70 cents a dozen for fertile eggs and a dollar delivery charge for each shipment of two to four crates. In Waycross the local supply was seasonal, so we imported eggs from Wisconsin. The cost included the current Chicago market price plus 20 cents service charge plus rail express. This amounted to about 90 cents a dozen delivered in Georgia. Fertility and hatchability in both cases were satisfactory.

Tissue cultures and media may be obtained from Microbiologic Associates, Inc., or from Difco Laboratories, Inc., or one of the other suppliers. One may obtain a cell line as (HeLa) or a primary culture such as (bovine kidney cells) at approximately 50 cents a tube. At Waycross we buy tube cultures and maintain a line of HeLa cells. It is, of course, cheaper on a large program to obtain tissues from a slaughterhouse and prepare the cultures yourself. This is the procedure we follow in Madison.

The next important item is a source of reference antigens and antisera. These may be obtained in lyopholized form and stored almost indefinitely. The usual sources of supply (Table 1) include the research laboratories at the experiment stations, the American Type Culture Collection and the commercial laboratories. Antibiotics are available from local drug outlets. A few items of specialized glassware such as tissue grinders, small serological instruments, forceps and scissors will facilitate work. Most laboratory supply houses have them.

With the materials on hand which can be stored indefinitely and knowledge of where other materials may be obtained when needed, one is equipped to do virological diagnostic work. From the standpoint of administration, we can divide the methods employed in virological diagnostic work in two classes, the rapid and the slow tests. In the rapid group, we place those tests that give results on the day they are initiated. This group may be subdivided into serological and histological methods. An example of the latter and one of the earliest methods ever employed in viral diagnosis is the demonstration of inclusion bodies in impressions or smears. Best known is the demonstration of Negri bodies for rabies by the use of Seller’s or Mann’s stain. Similarly, inclusion bodies for fowl pox, laryngotracheitis and distemper can sometimes be demonstrated by using the appropriate tissue; skin lesion for fowl pox, tracheal scraping for laryngotracheitis, and bladder scraping for distemper. The limitation of a histological diagnosis is that it is useful only if the result is positive, and also that a considerable amount of skill is required both in preparing material and in examining the material. Most laboratories, in which histological demonstrations are used, find it desirable to include
Once or twice a year a known positive. This tends to reenforce the skill of the individual who is doing the microscopic examinations.

The second group of rapid techniques are serological ones. Best known of this group is the hemagglutination test for Newcastle disease. Newcastle disease antigen is contained in the lungs of infected hens, and may be demonstrated by extracting lung tissue fluid. The tissue fluid may be diluted with physiological saline in the usual manner, washed chicken red blood cells added, and the hemagglutination read. Known immune sera inhibits the hemagglutination, and normal sera does not. Positive inhibition is diagnostic. Similar in principle is the complement-fixation test for vesicular stomatitis and foot-and-mouth disease. The antigens in this case are obtained from the fluid of vesicles on the muzzle or tongue of infected cattle. Known positive immune sera are employed to identify the complement-fixation reaction.

Some serological procedures fall with the rapid group, others with the slow. The complement-fixation test is among the rapid tests, and may be used to detect a rise in antibody titer. The hemagglutination test may be also

<table>
<thead>
<tr>
<th>General Sources</th>
<th>Special Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Type Culture Collection, Washington, D. C.</td>
<td>Supplies 155 virus different cultures, no antisera—10 dollar charge.</td>
</tr>
<tr>
<td>Communicable Disease Center, Atlanta, Ga. Rocky Mt. Laboratory, Hamilton, Montana</td>
<td>Supplies certain viruses and antisera on special request. No charge.</td>
</tr>
<tr>
<td>Agricultural Research Service, Beltsville, Md. Plum Island Animal Disease Laboratory, N. Y.</td>
<td>Supplies antisera to certain exotic viruses on special request. No charge.</td>
</tr>
<tr>
<td>Repository Newcastle Disease Virus Department of Veterinary Science University of Wisconsin, Madison.</td>
<td>Supplies 110 strains of NDV and limited antisera on special request. No charge.</td>
</tr>
<tr>
<td>Repository infectious bronchitis virus College of Veterinary Medicine Michigan State University, East Lansing.</td>
<td>Supplies strains of 1B and limited amounts of antisera on special request. No charge.</td>
</tr>
<tr>
<td>Agriculture Exp. Station Laboratories Medical and Veterinary Colleges.</td>
<td>Supply strains of virus and antisera that research staff have in available form. No charge.</td>
</tr>
<tr>
<td>Commercial Laboratories: Lederle Labs., Pearl River, N. Y. Markham Laboratories, Chicago, Illinois.</td>
<td>Supply listed strains of viruses, as living vaccines, or as non-living antigens, and listed antisera. Charge range from 5 to 50 dollars.</td>
</tr>
</tbody>
</table>
used in this way if the serum is available from convalescent animals. It is then called HI test, and used to identify the disease of Newcastle of chickens, influenza of swine and/or parainfluenza of cattle. One should ordinarily employ acute and convalescence sera obtained 10 days apart. If a presumptive diagnosis is desired, sera may be taken from representative animals in a flock or herd (test at least 10), and if some, but not all sera possess antibody and the history is indicative, a provisional diagnosis is possible.

A rapid test combining both histological and serological techniques, and, unfortunately, requiring expensive equipment not available in most diagnostic laboratorics, is a fluorescent antibody test. Tissues from an acutely infected animal obtained as a frozen section or as tissue impression are stained by flooding with fluorescent antibody. Slides are examined with ultra-violet microscope. Some spectacular successes have been obtained by this method, and it is probable that in the future the necessary equipment and materials will become available in many diagnostic laboratories.

The second group of tests can be called the slow tests. These procedures take more than one day, and may take several weeks. All of the isolation methods fall into this group. I will discuss first the use of laboratory animals—rabbit, mouse, guinea pig and chicken for recovery of the etiological agent. Laboratory animals provide several advantages over the other two host systems to be discussed. (1) They tolerate bacterial contaminants by acting as biological filters, (2) signs they exhibit are often diagnostic, and (3) the animals are usually readily available. The biggest drawback is cost, not only of the animal, but particularly the cost of holding and feeding. The route of inoculation is very important in diagnostic evaluation. This can be illustrated by using chickens and five avian viruses—laryngotracheitis, fowl pox, Newcastle disease, bronchitis and CELO viruses (Table 2). An

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sinus</th>
<th>Feather Follicle</th>
<th>Cloaca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newcastle disease</td>
<td>(rales) (CNS)</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>(rales)</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>CELO</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>Laryngotracheitis</td>
<td>swelling</td>
<td>_______</td>
<td>reddening</td>
</tr>
<tr>
<td>Fowl pox</td>
<td>swelling</td>
<td>pustules</td>
<td>(reddening)</td>
</tr>
</tbody>
</table>

agent suspected of being one of these viruses is introduced into three sites, into the sinus, rubbed into feather follicles, and rubbed into the cloaca. The following results would be indicative. Laryngotracheitis will produce a swelling in the sinus and a reddening of the cloaca, no apparent change in the feather follicles. Fowl pox will produce a swelling of the sinus and pustules in the feather follicles. Newcastle disease and bronchitis will not produce changes in the sinus, in the follicles or the cloaca, but they will usually induce
rales or other respiratory response. Newcastle, but not bronchitis, may induce a fatal neurotropic infection. CELO virus will not produce an observable sign by any of the three routes, and will not induce respiratory or neurological signs. It is possible with only two birds to obtain a presumptive differential diagnosis among five relatively common virus infections of poultry.

The embryonating egg is a particularly useful medium for the growth of viruses. Virus induced death of the embryo may occur as early as 24 hours or as late as 12 days. Some viruses do not produce death but cause lesions, such as pox on the membrane, dwarfing, distortion or congestion of the embryo. A few viruses grow without inducing any apparent change. They may be detected by demonstration of the antigen. The age of the embryo and the route of the inoculation are important. Many viruses may be inoculated into nine- or 10-day embryos by the allantois sac. Some viruses and rickettsiae are inoculated into the yolk sac of six- to eight-day-old embryos. Some viruses are grown upon the dropped choriolallantotic membrane. The inoculum for the embryo is usually prepared by centrifuging and adding antibiotics to the supernate. Adapted from Methods for the examination of poultry biologics. Table 3 illustrates the various manifestations following allantoic introduction of 10 known poultry viruses.

**TABLE 3**

*Differential Diagnosis of Poultry Viruses in the Chicken Embryo*

*Inoculation by Allantoic Sac.*

<table>
<thead>
<tr>
<th></th>
<th>Percent Mortality</th>
<th>Days to Death</th>
<th>Embryo</th>
<th>Lesion Special</th>
<th>OAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fowl Plague</td>
<td>90-100</td>
<td>2-4</td>
<td>cong</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>N virus</td>
<td>90-100</td>
<td>3-5</td>
<td>cong</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>NDV GB</td>
<td>90-100</td>
<td>2-4</td>
<td>cong</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>B1</td>
<td>50-100</td>
<td>4-8</td>
<td>cong-stunt</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>CELO</td>
<td>90-100</td>
<td>3-5</td>
<td>cong-stunt</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>0-100</td>
<td>2-6</td>
<td>cong-stunt</td>
<td>necrotic kidney</td>
<td>___</td>
</tr>
<tr>
<td>Laryngotraceitis</td>
<td>50-100</td>
<td>4-8</td>
<td>___</td>
<td>mottled liver plaques</td>
<td></td>
</tr>
<tr>
<td>Fahey-Crawley</td>
<td>50-100</td>
<td>4-8</td>
<td>___</td>
<td>necrotic kidney plaques</td>
<td></td>
</tr>
<tr>
<td>Fowl pox</td>
<td>0-50</td>
<td>4-8</td>
<td>___</td>
<td>splenomegaly plaques</td>
<td></td>
</tr>
<tr>
<td>Epidemic Tremor</td>
<td>0</td>
<td>___</td>
<td>___</td>
<td>muscular dystrophy</td>
<td></td>
</tr>
</tbody>
</table>

The third medium in which viruses can be grown are the tissue culture preparations. For small laboratories it is usually most practical to buy prepared cultures. The inoculum is prepared as it is for inoculation of chicken embryos. The culture is incubated and examined for cytopathogenic
effects. If one has never done tissue culture work, it would be desirable
to study a manual and then obtain several tubes containing tissue cultures
and a known cytopathogenic virus. Experiment with the system until you feel
that you can differentiate true cytopathogenic effects from other changes.
Tissue culture methods are particularly useful in diagnosing several of the
viral diseases of cattle. The agents of bovine rhinotracheitis, parainfluenza
and mucosal disease can be propagated in tissue culture. The swine enterovi-
ruses have been propagated only in tissue culture. Many of the agents that
can be propagated in eggs also can be propagated in tissue culture. It is
only a question of time before most laboratories engaged in general diagnostic
service will use tissue culture.

I have commented briefly upon the three media in which viruses may be
isolated. The same materials may be used for virus neutralization tests to
identify the agent that has been isolated or the antibody from a convalescent
animal. It is obvious that in most instances, eggs or tissue cultures would be
preferred for neutralization tests.

The virus neutralization test is performed by first mixing known immune
serum and the virus to be identified, or the serum to be tested and the known
virus. The mixture is inoculated by proper route. If the reaction is specific,
no neutralization; if the reaction is not specific, neutralization has occurred.
Virus neutralization test is dependable but not fool proof. We use it in
epizootiological surveys as nonspecific neutralizing-substances are not as
common as are HA inhibitors and anticomplementary substances. However,
when using a variety of wildlife sera, we prefer to report the presence of
neutralizing substances rather than specific antibody. Subsequent study usually
reveals if the substances are antibody.

In summary I have classified serological procedures from the viewpoint
of the diagnostic laboratory (Table 4). The tests you employ will depend

<table>
<thead>
<tr>
<th>TABLE 4</th>
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<tbody>
<tr>
<td><strong>Serological Procedure for Viruses as Viewed by a Veterinary Diagnostician</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serological Test</th>
<th>Low Cost Price of Materials and Technician Time</th>
<th>Simplicity Training Required to Obtain and Retain Competence</th>
<th>Applicability Wide Virus Spectrum</th>
<th>Dependability Absence of Non-Specific Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross protection</td>
<td>*−</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Virus neutralization</td>
<td>−</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Complement fixation</td>
<td>++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Hemagglutination inhibition</td>
<td>++</td>
<td>++</td>
<td>−</td>
<td>+++</td>
</tr>
<tr>
<td>Precipitation in gel and capillary</td>
<td>++</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>tube precipitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Possesses attribute wholly ++ in part + not at all −.
upon your evaluation of the four criteria. If the laboratory is small, and the work load diverse, simplicity of test will be the overriding consideration. If the laboratory is large, and the volume heavy, cost of the test will be foremost.

My purpose has been to emphasize the similarity of virological and bacteriological diagnostic procedures. Some of the laboratory methods for the diagnosis of viral infections can be utilized by any diagnostic institution.

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THE DIAGNOSTIC LABORATORY AND THE ECOLOGY OF ANTHRAX

G. B. VAN NESS, D.V.M.*

Washington, D. C.

The diagnostic laboratory has an important role to play in the control of anthrax in livestock. The differential diagnosis of anthrax from other concurrent infections is a continued problem. As the first losses in anthrax outbreaks are often unrecognized, it is important that the laboratory be familiar with when and where anthrax is likely to occur. The laboratory is in an excellent position to participate in the epidemiological studies, and to maintain a continuity of anthrax records. The laboratory could direct these studies.

Experience in recent years indicates anthrax outbreaks “develop” rather than “spread.” This play in semantics is quite important in understanding an outbreak and in establishing control procedures. This means that further losses may be expected on environments similar to the premises where the diagnosis has been made, but many farms in the community may have no need for control methods nor restrictions imposed on the affected area. A simplification of the factors associated with anthrax outbreaks is a formula:

\[
\left( \frac{\text{water}}{\text{grass}} + \text{drought} \right) \times \left( \text{soil pH 6.0} + \right) \times \left( \text{min. temp. 60° F.} + \right)
\]

Epidemiological data suggests food-borne or other causes may be suspected when this formula is not complete.

An evaluation of the anthrax potential environment requires information regarding the variety of ecological situations associated with losses. State and federal studies have been conducted toward this understanding since 1957. While some of this knowledge was useful in 1957, the variety of different yet similar situations favorable for anthrax had not then been demonstrated.

To evaluate the role of environment, a guide called “The Anthrax Report” was introduced to Regulatory Authorities in 1958. This guide has shown its value both as a means of reporting the situation and reducing the bulk of permanent records associated with anthrax losses. The insidious spread of anthrax should alert us for even more serious outbreaks in the future.

As normally employed, “The Anthrax Report” is mimeographed. For records, a Key sheet may be prepared, and permanent data recorded on 5” x 8” file cards. “The Anthrax Report” is as follows:

* Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
THE ANTHRAX REPORT

Location of the anthrax outbreaks.
1. Name and home address of each owner.
2. Location of the farm(s) on which anthrax has occurred.
3. Direction in miles from the nearest town.
4. Date of first loss from anthrax on each premise.

Make certain the location indicates the part of the farm where the anthrax occurs, if not the home address. This is important in epizootiological studies involving terrain and soil.

Select appropriate answers to the following:

A. Diagnosis of anthrax, how made.

1. Clinical evidence, based on previous losses on the farm or external symptomatology, no necropsy or other data of a more positive nature.
2. Clinical evidence, supported by necropsy data which could demonstrate spleen enlargement, edema in tissue or other suggestive lesions of anthrax.
3. Microscopic study, spore-forming bacilli present in a blood film; position of spore central, and rods with square ends; capsule may be present.
4. Laboratory study, including cultural and guinea pig inoculations for the identification of the anthrax organism.

B. Species and number of animals of each species reported dead from anthrax.

bh—Horses
bc—Cattle
bs—Sheep
bg—Goats
bp—Swine
bd—Dogs and cats and other farm pets
bz—Zoo animals or fur-bearing animals raised commercially

Following the code letter of each species involved, give the number of dead animals.

C. Human cases observed.

1. A human case not having direct contact with an animal dead of anthrax. This would include food-borne cases, or those having contact with persons working with diseased material.
2. A human case involving a person as a contact with diseased animals not suspecting anthrax.
   a. Person was a layman.
   b. Person was a professional.
3. A human who became infected while examining known or suspected anthrax material, including carcasses.
D. Animals shipped or sold which might be infected with anthrax.
   1. To another farm.
   2. To a sale ring or other distributing point where a change in ownership has occurred.
   3. To a packing plant or stockyard not having veterinary inspection.
   4. To a packing plant or stockyard having state or federal veterinary inspection.
   5. Other movements—give details.

E. Epizootic data.
   1. The area having losses has had experience with anthrax before, and there is no public concern.
   2. This is the first case of anthrax in an area not known to have anthrax. The public is unaware or unconcerned about the loss.
   3. The anthrax losses have caused much public concern, and forced sale of livestock has begun.
   4. This outbreak has some unusual characteristics, and an extensive investigation by anthrax experts is desirable.
   5. Not epizootic—give details.

F. The food supply.
   1. Grass fed—no supplemental feed.
   2. Feed is hay, silage, chopped forage, or other vegetation which may have come from an anthrax contaminated area.
   3. Food supply includes commercial feed. This commercial feed includes animal by-products. If anthrax is suspected in commercial feed, impound the suspected feed. Make a report to the feed manufacturer and invite a representative of the company to participate in further studies. This group includes meat purchased to feed fur animals and zoo animals.
   4. The outbreak is not due to a contaminated food supply. It is due to faulty immunization or other atypical factors. Give details.

G. Terrain on which the animals feed.
   1. Well drained hill pasture.
   2. Well drained rolling pasture.
   3. Poorly drained soil, some evidence of water damage on grass.
   4. River or stream bottom land.
   5. River or stream bottom land, recently flooded.
H. Water supply.
   1. From wells with sanitary tanks or a piped water supply.
   2. Ponds with sanitary tanks.
   3. Streams, steady flow of water.
   4. Streams, intermittent flow of water.
   5. Ponds.

   Note: The conditions of the water supply should cover not more than one month preceding the outbreak.

I. Insect and other vector situations.
   1. No insect problem exists.
   2. House flies are present.
   3. Horn flies, stable flies are a problem.
   4. Horse flies or deer flies are active in the area.

   Note: If horn flies or stable flies are a problem, or there are other insect problems which can be corrected recommend an insect control program.

J. Program of control to be followed.
   1. No activity such as vaccination or area quarantine is indicated at this time.
   2. There is a premise quarantine and vaccination.
   3. An area quarantine and vaccination program is indicated.
   4. The following counties or portions of these counties will be quarantined because of the threat of anthrax. Give details.

INFLUENCE OF TEMPERATURE AND MOISTURE ON ANTHRAX

Interest in temperature and moisture related to anthrax outbreaks is such that a record of the high and low temperatures, and measured local rainfall has considerable epidemiological significance. It is commonly observed that a deviation from normal weather is associated with anthrax outbreaks. The investigator should obtain from the nearest weather bureau observer, a record of the actual high and low temperatures for the past two months, the average high and low temperatures for the corresponding days, and the daily measured rainfall during the period of observation. The nearest newspaper office can provide the name of the local weather observer.

GEOLOGICAL FORMATIONS AND SOILS ASSOCIATED WITH THE OUTBREAK

The investigator of the anthrax outbreak should determine the extent of the soil area to which the affected animal has been exposed during the last
month. Normally, this would be the pasture or feed lot in which the herd is confined. Soil conservation representative, county agent, or other person familiar with soil classification can assist in a soil study of the premise on which the losses occurred, and of other areas thought related to the outbreak. Food-borne anthrax, due to contaminated fodder, hay, or other harvested and prepared foods, may be difficult to trace, but efforts in this direction are worthwhile.

Lacking specific soil classification information, the investigator should prepare a detailed map, even if quite rough, covering his impression of the environment in which the anthrax may be endemic. The following classifications may serve to cover the varieties of environments encountered in studies of anthrax outbreaks.

A. Bottom land and alluvial soils.

1. River bottoms.
   a. River recently overflowed portions of the farm, grass is injured by water, mud covers the grass, or weeds have replaced the grass.
   b. Dikes prevent the river from overflowing the area, rain water stands in lowland following rains, grass is injured by standing water, cracked soil or mud found in pasture.
   c. Pasture consists of woods and wood's grasses, reeds and tall weeds; no short grass or pasture grasses in the woods.

2. Marsh land and alluvial bottoms influenced by salt water or brackish water.
   a. Marsh grass subject to overflow by salt water at high tide or during storms.
   b. Marsh grass originally influenced by salt water now diked, or otherwise fresh water marsh.

B. Cultivated land.

1. Sandy land, well drained, not subject to overflow or standing water.
2. Sandy clay land, well drained, no standing water, alkali spots or clay pan.
3. Loam, peat or muck soil. Obtain pH of this soil.
4. River valley land, rich organic soil, not subject to flood.
5. River valleys, normally cultivated or hay crops, not subject to overflow except in high floods.
6. Rich organic soils, derived from glacial wash, stream silt, or dust.
7. Prairie grass land, no distinctive features.
8. Alkali spots, slick spots, buffalo wallows, playas, or other unusual conditions of local nature occurring on uplands, which may hold water during wet weather, and become dusty and dry during hot weather.
C. Rough land, used for pasture or timber.

1. Well drained sandstone or acid shale land, no alkali bloom or evidence of alkali at any time.

2. Sandstone, shale and limestone interbedded, in regions of high rainfall, well drained, stream beds with clean gravel, no slime on rocks or in water.

3. Sandstone, shale and limestone, growth of grass excellent, water course with grass near water, and rocks covered with slime.

4. Glaciated region, rough land with pot holes, swamps, bogs or small lakes.

5. Bluffs, with wet spots and good grass growth at their base, evidence that cattle use such areas for grazing and watering.

D. Hill or mountain areas, steep slopes and small, well drained mountain meadows.

1. Mountain pastures, well drained grass land, subject to dry periods and short grass.

2. Mountain pastures, grass shaded by trees or on north slopes with good growth indicative of a good water supply much of the year.

3. Mountain pastures, with landslide areas, bogs in elevated valleys, ponds formed by landslides, or valley washes with persistent bogs or ponds.
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